BIOLOGICAL RESEARCH FOR NURSING

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BIOLOGICAL RESEARCH for NURSING

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Moving Biological Nursing Science into the New Millennium

Patricia A. Grady, PhD, RN, FAAN

The beginning of a new journal, *Biological Research for Nursing*, is an important and timely step in the evolution of nursing science. Ours is a field that is concerned with the biopsychosocial focus on the individual. Nursing science spans the continuum from basic biological to clinical and applied research. We have an unparalleled opportunity in our field to carry scientific inquiry from the bedside to the bench and back to the bedside.

The question is often raised, "Do nurses do biological basic research?" Or sometimes, "Why do nurses do basic or biological research?" There are many answers to that question. Primarily, the questions that nurses ask are those that are framed from a clinical perspective. The inquiry may be basic or biological, but central to this picture is a clinical problem or issue. As we approach the 21st century with its increase in technology and accompanying increase in knowledge, it is increasingly important that nurses be able to interface effectively with other health team members and assume a leadership role. This nursing perspective is essential for the ultimate welfare of patients and families.

There is often a great deal of biological basic science information to incorporate when partnering in multidisciplinary research teams. Whether the nurse is a researcher or a clinician involved in the studies, it is important that there be a level of understanding and an ability to interpret the findings that will ultimately be incorporated into his or her care delivery. Whether the research occurs in an acute care setting, high-technology setting, telehealth setting, or an environment of genetic counseling, it is important that we are major participants in the new science that will affect the health of our nations' people.

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Nurse researchers in the basic sciences are making major contributions to science in a variety of ways. They are facilitating practitioners in clinical practice, participating on study sections of the National Institutes of Health, and coordinating and participating in diverse multidisciplinary panels in nursing and other schools, such as medicine, public health, and epidemiology, to name a few. They are participants in the industrial sector. These nurse scientists are publishing outside their field as well as within nursing journals and are serving as mentors for pre- and postdoctoral fellows.

Let me, for a moment, address the growth of biological nursing science at the National Institute of Nursing Research (NINR). In the early days, these studies were within one portfolio. Now they are distributed among all the portfolios, and the investigators are using many different mechanisms. These studies are having an impact in advancing not only nursing science but also science of a multidisciplinary nature— something very important in nursing. Nurse researchers are now being funded across the career trajectory from predoctoral fellows to postdoctoral fellows, to longstanding research project grants.

The majority of the research funded by NINR is clinical in nature. The basic research accounts for a smaller percentage of the science that we fund, but the contribution that it makes is unique and important.

Scientific areas being addressed by basic biologic researchers include women's health; respiratory, cardiovascular, and neurosensory problems; wound healing; technology issues; and changes in muscle in various states of health and illness. Scientific areas of opportunity that are highlighted at NINR also accommodate basic science studies. Recent examples include psychoneuroimmunology; gender differences in pain responses; and genetic research, as it moves from the bench to the bedside.

I would like to commend the founders of this journal, Dr. Patsy Perry and Dr. Chris Kasper, as well as the editorial board and the many others who will be contributing a high level of science to this journal. We, in the nursing community, look forward to participating in the dialog directly as contributors or indirectly as we benefit from the results of talented researchers who will be publishing in this journal. *Biological Research for Nursing* fills an important gap in our library of nursing journals.

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The Effect of Familiarity on Distraction and Single Cue Use after Hippocampal Damage

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Spatial disorientation frequently occurs in conditions such as Alzheimer's disease that damage the hippocampus, a brain structure necessary for learning and memory. Use of a single cue to mark a submerged escape platform in the Morris water test can reduce spatial disorientation in rats. If the cue used is a familiar one, disoriented rats perform the wayfinding task as well as control animals. However, in a real-world environment, cues rarely occur alone. This study examined whether rats with bilateral hippocampal lesions familiar with a cue performed the Morris water test as well as controls and faster than lesioned rats unfamiliar with the cue when a distracter was present. Bilateral electrolytic lesions were made in male rats that had received either familiarity with a cue or handling only. Familiar lesioned rats were introduced to the distracter on test days 1 (FB1) or 3 (FB3), and unfamiliar lesioned rats on day 3 (UB3). No significant differences were found for FB1 or FB3 rats and their respective controls. FB1 rats increased mean swim times on day 1 compared to preoperative day 4 (16.44 ± 4.3 vs. 4.06 ± 2.1 s, respectively, $p < 0.03$) but quickly adapted to the distracter. FB3 rats were slower than FB1 rats on day 3 (6.81 ± 1.0 vs. 4.56 ± 0.3 s, respectively, $p < 0.05$). UB3 rats were impaired on the task compared to FB1 rats on days 3-7 ($p < 0.05$). These findings suggest that cue familiarity is effective in the presence of a distracter and that the response of disoriented rats to a distracter is influenced by the amount of prior experience with the cue.

Key words: *Cue, disorientation, wayfinding, hippocampus, distraction, familiarity*

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One of the main concerns in nursing is the manipulation of the environment to assist people in adapting to changes in their health status. The relationship between individual and environment has been central to nursing since Nightingale (1969) first described the profession of nursing in the 19th century. Spatial disorientation, in which a person becomes lost in a known environment or is unable to learn about a new environment, is an altered health state that may be amenable to environmental manipulations. Nurses are often the primary care providers for disoriented clients and may struggle to provide therapeutic interventions to assist their disoriented clients. Understanding how the brain processes information about an environment is critical to developing such interventions.

The ability to navigate in a large-scale physical environment is a basic cognitive function necessary for survival. Normally, navigation is done through the formation of cognitive maps that encode information about the relationships among multiple objects in an environment (O'Keefe and Nadel 1978; Hetherington and Shapiro 1997; Martin and O'Keefe 1998). The hippocampus, a large structure in the temporal lobes of the brain, is required for the formation and use of cognitive maps (Taube 1998). Hippocampal damage often

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occurs in Alzheimer's disease, stroke, head injury, or AIDS-related complex and leaves affected persons with spatial disorientation (Pigott and Milner 1990; Heston and White 1991; Maguire and others 1996). The use of a single visual landmark, or cue, has been shown to reduce spatial disorientation in rats and pigeons with damage to the hippocampus (Morris and others 1982; Speakman and O'Keefe 1990; Fremouw and others 1997) and in humans who are disoriented (Bird and others 1995). Cue use, a function of the parietal lobe, involves only the relationship between the individual and the cue itself (O'Keefe and Nadel 1978). As long as the cue is in visual range, the person remains oriented. But if the person loses sight of the cue, disorientation occurs. Methods that optimize cue use are valuable in the prevention of disorientation in persons that depend on the use of a single visual cue to remain oriented.

Early studies demonstrated that preoperative familiarity with a single cue enhances its use in preventing disorientation in wooden mazes (Winocur 1982; Jarrard and others 1984). However, questions have been raised regarding the validity of mazes as a measure of large-scale space because they allow only left and right turns at choice points (Winocur 1982). The Morris water test, a large tank in which animals swim to locate a hidden escape platform, does not have the same validity concerns because animals are able to make multiple turns at any given location in the test. Recently, we reported that rats familiar with a cue prior to receiving hippocampal lesions performed a spatial task as well as controls following hippocampal damage when the cue was the only object in the Morris water test (Holden and Therrien 1993). However, a cue rarely exists alone in an environment. Other objects in that environment may distract the attention of the individual from the cue, causing the person to become disoriented and lost. Although there are studies that report the use of a distracter in wooden mazes (Raphaelson and others 1965; Riddell and others 1969; Xavier and others 1990), there are no studies to date that examine the outcomes of a distracter in the Morris water test.

Distraction and Familiarity

Environmental familiarity may be defined as repeated experience with the elements and contexts of a place, such that a cognitive map is formed in memory. A person can use this map to distinguish minor differences that exist in an environment, so that any changes that occur will not disrupt smooth, safe passage through that place, a behavior known as wayfinding.

When a novel element is added to a familiar environment, healthy humans and animals tend to notice the element, arrest activity, turn toward the novel object, and then explore it (O'Keefe and Nadel 1978). The adaptive value of this response, termed distraction, is the need to remain familiar with that environment. Once the novel item becomes familiar and the cognitive map is updated, the individual or animal returns to the earlier wayfinding task.

In spatial disorientation, the adaptive roles of familiarity and distraction are lost. Affected persons may wander, oblivious to environmental changes, or they may become extremely distractible and respond excessively to any environmental stimulus (Martino-Saltzman and others 1991). In both cases, the ability to become familiar with a place is disrupted.

In rats, it has long been known that the hippocampus is needed to detect changes in an environment because selective hippocampal cells fire when mismatches occur between what is in memory and what is in the world (McNaughton and others 1983; Samsonovich and McNaughton 1997). When mismatches are encountered, as when a goal in a maze is changed to a new location, a rat is stimulated to explore, find the goal, and update the memory. Rats with hippocampal damage explore less than do controls, returning to the previous goal location long after the goal has been moved (Savé and others 1992). Although such findings appear to argue against distractibility in rats with hippocampal damage, in familiar environments general activity may actually increase in the presence of environmental stimulation, even if that stimulation is not novel (Hall 1956, cited in O'Keefe and Nadel 1978). This increase may be similar to the excessive distractibility seen in humans with spatial disorientation.

The response to novel objects by animals with hippocampal damage in a cue task is not known. O'Keefe and Nadel (1978) suggested that dependence on a cue for wayfinding limits responses to environmental change. This is reasonable because the animal must attend to the cue to remain oriented. Consequently,

distraction may be detrimental to the performance of a cue task.

In studies that investigated the effect of distraction on a cue task, the results are equivocal. Rats with hippocampal damage have been shown to respond to a distracter in a maze, though less than controls do; however, when the hippocampal lesion was extensive, rats showed no response to the distracter (Raphaelson and others 1965; Riddell and others 1969). In contrast, Xavier and others (1990) reported that rats with dorsal hippocampal lesions were as distractible as controls to a novel white card on a runway maze. In a large-scale cue task, such distractibility could result in disorientation.

Studies that examine the responses of rats with hippocampal damage to a distracter when they are familiar with a cue have not been reported. This study tested the effect of familiarity on single cue use following bilateral hippocampal damage when a distracter was introduced into the testing environment. A rat model of spatial disorientation was selected for 2 reasons. First, rats with hippocampal damage show physiological and behavioral similarities to human spatial disorientation (Scoville and Milner 1957; Morris and others 1982; Corkin 1984; Speakman and O'Keefe 1990). Second, a rat model allows for control of the extent of damage to the hippocampus and reduces damage to other brain structures, such as the parietal lobe, that may interfere with optimum cue use (Kolb and Walkey 1987). Based on existing knowledge, the hypotheses for this study were (1) rats with bilateral hippocampal lesions familiar with a single cue will perform a cue task as well as control rats in the presence of a distracter and (2) rats with hippocampal lesions familiar with the cue will perform the cue task more efficiently than rats with hippocampal lesions unfamiliar with the cue.

Materials and Methods

Sample

Forty-seven male Sprague-Dawley rats, weighing 250-350 grams at the time of surgery, were used. All animals were housed individually with free access to standard rodent feed and water. The animals were maintained on a reverse light-dark cycle (lights on at 8:00 PM and off at 8:00 AM). Testing took place during the dark cycle habituation for the animals.

Testing Apparatus

The testing apparatus was an adaptation of the Morris water test, a commonly used measure of large-scale wayfinding (Holden and Therrien 1993). An animal swims in a large tub to locate a platform, 6 inches in diameter, submerged and invisible beneath the water's surface, a task that requires a functioning hippocampus. If the hidden platform is marked by a single cue and moved to a new location in the tub each trial, the animal must develop nonhippocampal strategies to locate the platform. Two dependent measures are recorded: swim time, or the time taken to locate the goal once the rat is placed in the water until its paws touch the platform, and directional heading, the point at which the rat commits itself to swim in a certain direction. Heading error is calculated by measuring the angle between the directional heading and a straight line between the platform and the starting point.

Preoperative Procedure

All rats received a preswim, during which they were placed in the water, allowed to swim for 10 s, and then placed on an unmarked platform to learn that an escape from the water was available. All rats were then randomly assigned to receive preoperative familiarity with the cue (familiar group) or handling only (unfamiliar group). The familiar group received training to the cue for 4 preoperative test days, 4 trials per day, a regimen that allows for optimum acquisition of the cue task (Holden and Therrien 1993). Each animal was allowed to swim for a maximum of 120 s. If an animal did not locate the platform within this time, it was removed from the water, placed on the platform, and given a swim time score of 120 s. The cue and platform were moved to 1 of 4 randomly assigned locations in the tub for each trial, ensuring that the animals were being trained to wayfind by associating the cue with the goal. Unfamiliar rats received a similar protocol, except that they were placed in the water for 10 s only and no cue marked the platform location.

Surgery

Following the preoperative sessions, rats were randomly assigned to receive either bilateral electrolytic lesions or sham surgery, for control animals. All animals were anesthetized with ketamine and xylazine (87/13 mg/kg). Using aseptic technique, a scalp incision was made and the muscle and fascia retracted. Burr holes were then made in the skull according to stereotaxic coordinates that allowed for 6 dorsal and 2 ventral penetrations made in each side of the hippocampus. Cathodal electrolytic lesions were made by passing current through a stereotaxically placed 250-micron diameter stainless steel wire insulated with Teflon. For dorsal lesions, 0.5 mm of Teflon coating was removed from the wire tip and 1.0 mA of current passed for 15 s for each electrode placement. For ventral lesions, 3 mm of the wire insulation was removed and 1.5 mA of current passed for 15 s. An anodal reference electrode was clipped to the wound edge. Control animals received sham surgery in which the electrode was lowered into the brain but no current was passed. Following lesioning, wound clips were inserted and the animal kept warm until awake. A recovery period of 4 to 7 days was allowed for healing of incisions before testing began.

Pilot data using 6 animals confirmed the location of lesion placement and the destruction of a minimum of 30% of hippocampal tissue, sufficient to disrupt cognitive mapping (Holden and Therrien 1993). Electrolytic lesions have long been used as a reliable method of destroying both neurons and fibers of passage (Gage 1985; DiMattia and Kesner 1988; Compton and others 1997; Fremouw and others 1997). This method was selected over the use of chemical lesioning with agents such as colchicine or kainic acid, which destroys only neurons, because chemical lesioning is more difficult to confine to a given brain area. Since the role of the hippocampus in spatial navigation is well documented, the destruction of fibers of passage was not an issue in the present experiments (O'Keefe and Nadel 1978; Morris and others 1982; Hetherington and Shapiro 1997; Martin and O'Keefe 1998).

Postoperative Testing

The results of a pilot study revealed that when a distracter was placed in the water test on the 1st postoperative day, rats in the unfamiliar group were actually being tested on an object discrimination task rather than a single cue task with a distracter present. It was determined that a more valid test of distracter effects would be to expose the unfamiliar groups to the single cue the first 2 days postoperatively, when the greatest amount of learning takes place (Holden and Therrien 1993), and then place the distracter in the testing apparatus. Thus, the unfamiliar rats had a chance to learn that the cue was associated with the escape platform before they had to decide whether to choose to go to the cue or to the distracter. To gain additional information regarding the effect of distraction, the familiar control and lesion groups were randomly assigned to receive the distracter beginning either on test day 1 or test day 3. For postoperative testing, a total of 6 groups were formed (Fig. 1). Four groups of animals were exposed to the single cue alone on the first 2 days postoperatively, 2 that had preoperative training to the cue, and 2 that did not, then the distracter was placed in the testing apparatus. Postoperative testing was done for a total of 7 days, 4 trials per day, which provided a minimum of 5 days exposure to the distracter. Two other groups of rats that received preoperative cue familiarity were exposed to the distracter on the 1st postoperative testing day. Distraction was defined as an increase in swim time and heading error.

The distracter was a metal can, approximately 10 cm high and 6 cm in diameter, white with 2 cm black horizontal stripes, rising out of the water. Both the distracter and the cue were randomly assigned to 1 of 4 different locations in the tub for each trial.

Histology

Following testing, all rats were anesthetized with sodium pentobarbital and perfused through the heart with isotonic saline followed by 10% neutral phosphate buffered formalin. The brains were removed and stored in 30% sucrose for approximately 1 week. The brains were then frozen and cut into 80-micron coronal sections on a sledge microtome. Every 4th section was mounted on a gel-coated slide, stained using cresyl violet, dehydrated through a series of alcohols and xylenes, and coverslipped.

The brain sections for each animal were analyzed to determine hippocampal lesion size as well as extra-

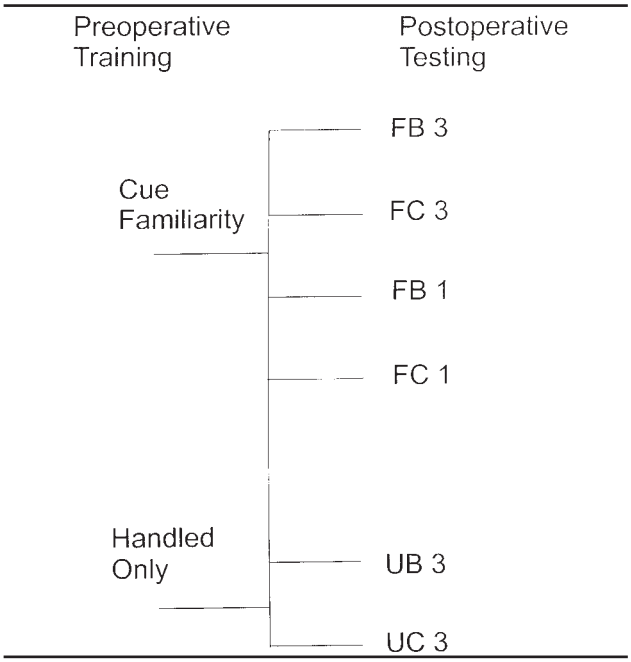

Figure 1. Preoperatively, rats were assigned to receive familiarity to the cue or handling only. Following bilateral hippocampal lesions or sham surgery for controls, the cue-familiar animals were assigned to receive the distracter on test days 1 or 3. The rats unfamiliar with the cue received the distracter on test day 3. A total of 6 groups were tested. Abbreviations: FC3 = familiar controls exposed to the distracter on test day 3; FB3 = familiar lesioned animals exposed to the distracter on test day 3; UC3 = unfamiliar controls exposed to the distracter on test day 3; UB3 = unfamiliar lesioned animals exposed to the distracter on test day 3; FC1 = familiar controls exposed to the distracter on test day 1; FB1 = familiar lesioned animals exposed to the distracter on test day 1.

hippocampal damage. Each section was projected onto a Numonics 2200 digitizing tablet using a Bausch and Lomb Tri-Simplex microprojector. The image was then traced with a magnetic puck connected to the digitizing tablet that transmitted grid data to an IBM computer. The Sigma Scan computer program was used to translate the grid data into area measured. In the hippocampal formation, the hippocampus proper, the dentate gyrus, alveus, and hippocampal fimbria were digitized. Comparisons of lesioned animals to unoperated controls were made to determine lesion size. Intact neocortex and thalamic nuclei located ventral and medial to the hippocampus were also digitized and compared to unoperated controls to determine levels of neocortical and thalamic damage.

Statistical Analysis

Profile analysis and univariate analysis of variance (ANOVA) were used unless otherwise noted. Profile analysis is similar to 2-way ANOVA with repeated measures. It tests parallelism of the involved profiles as the interaction effect; a levels hypothesis, or that the profiles are the same level (row main effect); and a flatness hypothesis, that the combined means of a pooled profile are equal (column main effect).

Results

Histological Analysis

No significant differences were found for extent of total hippocampal damage or for dorsal or ventral lesion extent across lesioned groups. Dorsal hippocampal damage averaged 61%, and ventral hippocampal damage averaged 47%. Three animals exhibited statistically significant motor aberrations, as shown by continually swimming in small diameter circles. These behaviors skewed both swim time and heading error, so these animals were excluded from the study. Microscopic examination of these brain sections revealed diffuse damage in the basal ganglia and internal capsule in 2 animals and damage to the ventrolateral thalamic nuclei in the 3rd animal.

Behavior of Control Rats— Evidence of a Distracter Effect

A total of 6 groups were tested in the present experiments. Familiar controls (FC3) and rats with hippocampal lesions (FB3) that were exposed to the distracter on test day 3 received 6 days of familiarity with the cue, 4 days prior to surgery, and 2 days after surgery. Unfamiliar controls (UC3) and rats with bilateral hippocampal lesions (UB3) that were exposed to the distracter on test day 3 had 2 postoperative days of familiarity with the cue prior to introduction of the distracter. Familiar controls (FC1) and rats with bilateral hippocampal lesions (FB1) introduced to the distracter on test day 1 had 4 prelesion days of familiarity with the cue.

Swim Time

Comparisons of control groups should demonstrate differences in behavior on the days the distracter was introduced into the testing environment if distraction occurred. Figure 2A shows that, as expected, the UC3 group took significantly longer to locate the cue on day 3 compared to FC3 rats, as shown by significantly increased swim times on day 3 (6.74 ± 1.0 s vs. 13.83 ± 2.3 s; $p < 0.05$; FC3 and UC3, respectively) and day 4 (7.31 ± 1.1 s vs. 13.19 ± 2.4 s; $p < 0.05$; FC3 and UC3, respectively).

Profile analysis of the FC1 and FC3 groups demonstrated a significant interaction effect, $F = 4.78(6, 7)$, $p < 0.03$. Univariate ANOVA showed that FC1 rats were distracted on day 1 as compared to FC3 rats (10.34 ± 1.6 vs. 4.63 ± 0.6 s, respectively, $p < 0.05$) and on day 2 (7.32 ± 1.0 vs. 4.62 = 0.4 s, respectively, $p < 0.05$). That FC1 rats were distracted is underscored by a significant difference between day 1 and subsequent test days as determined by paired *t*-tests. Mean swim times for day 1 (10.34 ± 1.6 s) were significantly longer than on day 3 (5.73 ± 0.9 s, $p < 0.09$), day 4 (4.73 ± 0.6 s, $p < 0.04$), day 5 (4.66 ± 1.0 s, $p = 0.01$), day 6 (4.15 ± 0.3 s, $p < 0.007$), and day 7 (4.54 ± 0.5, $p = 0.02$). Similar differences were found between day 2 and days 4-7 (Fig. 2A). In addition, FC3 rats showed evidence of distraction when the distracter was introduced, as mean swim times increased on days 3 and 4 as compared to day 1 (4.63 ± 0.6 vs. 6.74 ± 1.0 s, days 1 and 3, respectively, $p = 0.06$) and day 4 (7.31 ± 1.2 s, $p < 0.04$).

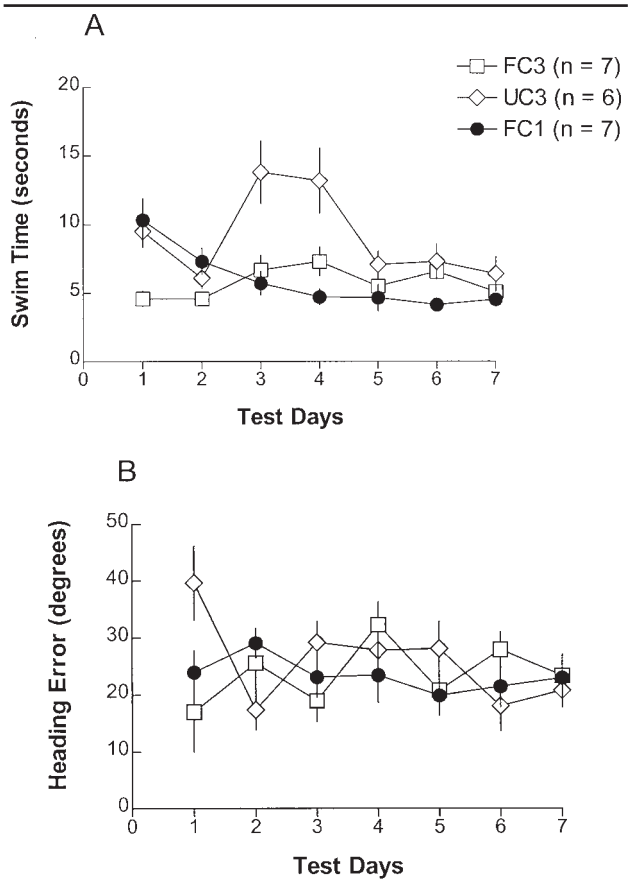

Figure 2. Mean swim times (A) and directional heading errors (B) for familiar (FC3) and unfamiliar (UC3) control rats initially exposed to the distracter on test day 3 and familiar control rats initially exposed to the distracter on day 1 (FC1). Mean ± SEM for sample sizes indicated on Figure. FC1 rats exhibited distraction on days 1 and 2 before decreasing swim time and heading error. FC3 rats exhibited mild distraction from day 3 through day 7. UC3 rats demonstrated a marked response to the distracter on days 3 and 4.

Heading Error

There was a great deal of variability in the day-to-day responses of the control groups across test days. Profile analysis showed that the profiles of the FC3 and UC3 groups were parallel, $F = 3.72(6, 7)$; $p = 0.05$, but no lesion effect or significant change over time between the two groups was seen. However, paired *t*-tests showed that the heading error of UC3 rats was significantly larger on day 3 when the distracter was introduced than on day 2 (29.21 ± 3.7 degrees vs. 17.38 = 3.5 degrees, respectively, $p < 0.007$, Fig. 2B), while remaining at about 28 degrees for days 4 and 5, only to decrease significantly on day 6 (28.13 ± 4.7 vs. 18.04 ± 4.3 degrees, days 5 and 6, respectively, $p = 0.03$). Interestingly, the FC3 group experienced their greatest heading error on day 4 (18.96 ± 3.7 vs. 32.29 ± 4.2 degrees, days 3 and 4, respectively, $p = 0.04$). Their heading error then decreased on day 5 (20.82 ± 3.5 degrees, $p = 0.02$).

Comparisons between the 2 familiar control groups for heading error showed parallel profiles, $F = 0.37(6, 7)$, $p =$ NS. As expected, there was no significant effect for lesion or for change over time. No significant differences were found with univariate ANOVA between the FC1 and FC3 groups on any test day.

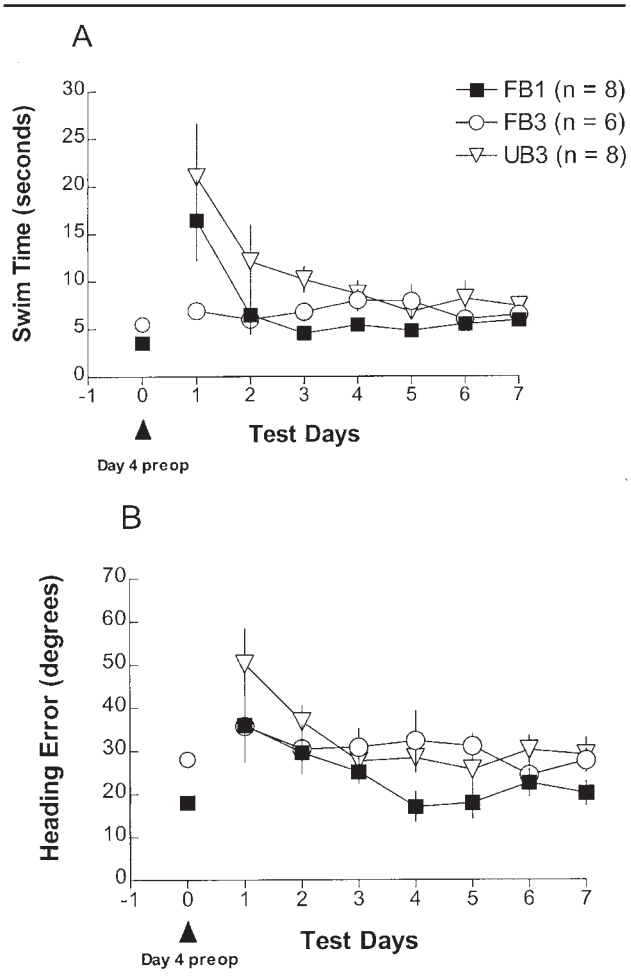

Figure 3. Mean swim times (A) and directional heading errors (B) for familiar rats with hippocampal damage first receiving the distracter on day 3 (FB3) or day 1 (FB1) and for unfamiliar rats with hippocampal damage first exposed to the distracter on day 3 (UB3). Means ± SEM for sample sizes indicated. FB1 rats evidenced distraction on day 1, as compared to the last day of preoperative training (A). FB3 rats responded to the distracter on day 3 for swim time and day 4 for heading error. UB3 animals did not appear to respond to the distracter.

The Effect of Distraction on Rats with Hippocampal Lesions

Swim time. Familiar rats with hippocampal damage also demonstrated responses to the distracter. Profile analysis showed that the profiles of the FB1 and FB3 groups were parallel, $F = 2.0(6, 7)$, p = NS. No lesion difference or change over time was seen. Univariate ANOVA with Scheffe post hoc tests demonstrated that FB3 rats were significantly slower in locating the cued platform on day 3 than FB1 rats (6.81 ± 1.0 vs. 4.65 ± 0.3 s, $p < 0.05$, Fig. 3A). No differences were found in comparisons of consecutive test days for the FB3 group.

Although it took FB1 rats approximately 10 s longer to locate the cued platform on day 1 when compared to FB3 rats, statistical significance was not obtained due to considerable within-group variance in the FB1 group. However, FB1 rats were faster in locating the platform on day 2 than day 1 (6.99 ± 2.1 vs. 16.44 ± 4.3 s, $p = 0.06$), suggesting a decrease in response to the distracter on day 2. Additionally, when comparing the last day of preoperative training with the 1st day of the postoperative testing period, a significant increase in swim time was obtained (4.06 ± 2.0 vs. 16.44 s, respectively, $p < 0.03$, Fig. 3A). Such a difference was not found with FB3 rats.

UB3 rats did not appear to respond to the distracter. These animals showed no increases in swim time on day 3 when the distracter was introduced. Nor did they show an increase in swim times on subsequent days of testing. Figure 4A compares the performance of the UB3 and UC3 groups. Profile analysis indicated that the profiles of the 2 groups were parallel, $F = 2.30(6, 7)$, p = NS. A lesion effect was seen, as the UB group took longer to locate the platform, $F = 6.09(6, 7)$, $p < 0.02$, but no significant change over time was seen. No significant differences were found between the 2 groups using univariate ANOVA.

Heading error. FB1 and FB3 heading errors were similar on day 1, and both groups decreased heading error on day 2 (Fig. 3B). The 2 profiles were parallel, $F = 0.042(6, 7)$, p = NS. No lesion differences were expected or found, and change over time was not significant. Univariate ANOVA revealed significant differences between FB1 and FB3 rats beginning the day after introduction of the distracter to the FB3 group, on day 4 (16.97 ± 3.6 compared to 32.29 ± 6.9 degrees, respectively, $p < 0.05$), and again on day 5 (17.88 ± 3.8 vs. 30.96 ± 3.0 degrees, $p < 0.05$).

No significant differences were found between consecutive days for FB3 rats, as heading error across days was fairly consistent (Fig. 3B). FB1 rats decreased heading error, as shown by differences

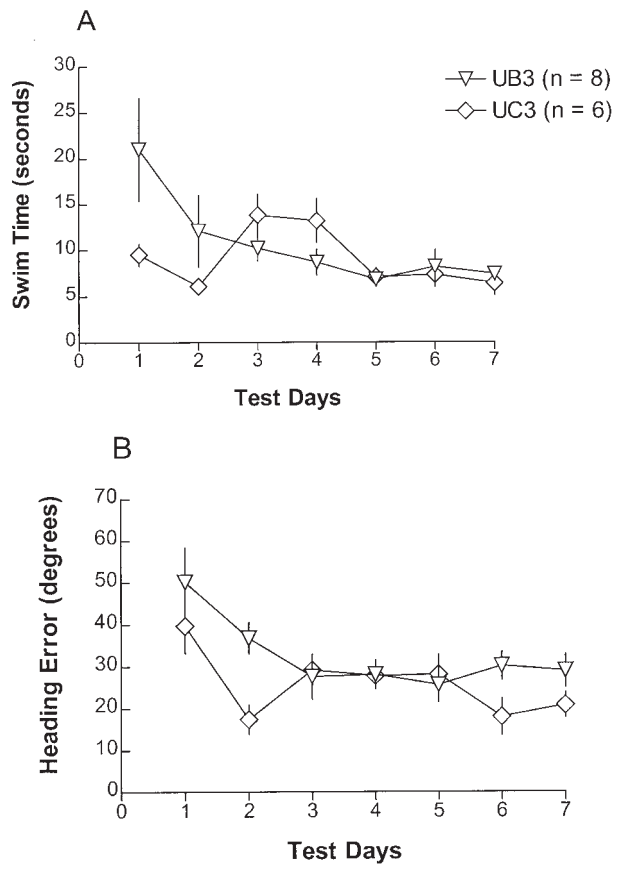

Figure 4. Mean swim times (A) and directional heading errors (B) of unfamiliar control rats (UC3 and unfamiliar rats with hippocampal lesions (UB3). Means ± SEM for sample sizes indicated. The effect of distraction in UC3 rats and a lack of effect in UB3 rats is evident.

between day 1 (35.94 ± 8.7 degrees) and day 4 (16.97 ± 3.6 degrees, $p = 0.07$) and days 1 and 5 (17.88 ± 3.8 degrees, $p < 0.03$). However, no significant differences were obtained between consecutive days. Additionally, no significant differences were found for either familiar lesioned group when comparing the last preoperative day of training with the 1st postoperative day.

UB3 rats did not show an increase in heading error following introduction of the distracter either on day 3 or consecutive test days. UB3 rats made significantly larger heading error than FB1 rats on day 4, which could be interpreted as an effect of distraction for the UB3 group (Fig. 3B). However, in the absence of differences between consecutive days for the UB3 group, it is likely that this difference was due to the effect of familiarity in FB1 rats.

Figure 4B compares UB3 and UC3 animals. Profile analysis indicated that the profiles for the two groups were parallel, $F = 3.80(6, 7)$, p = NS (Fig. 4B), and a lesion effect was found, with UB3 animals demonstrating greater heading error, $F = 3.50(6, 7)$, $p = 0.06$. Univariate ANOVA demonstrated that UB3 rats had greater heading error than controls on day 2 (36.78 ± 3.7 vs. 17.38 ± 3.4 degrees, respectively, $p < 0.003$) and on day 6 (30.06 ± 3.4 vs. 18.04 ± 4.2 degrees, $p < 0.05$).

The Effect of Familiarity on Rats with Hippocampal Damage

Swim time. To determine whether the effect of familiarity remained when a distracter was added to the testing environment, rats with hippocampal damage familiar with the cue were compared to their respective control groups. Profile analysis for the FB3 and FC3 groups showed that the 2 profiles were parallel, $F = 0.75(6, 6)$, p = NS, and there was no effect of lesion and no change over time. These findings were supported by univariate ANOVA. With the exception of day 1 (6.91 ± 0.71 vs. 4.63 ± 0.58 s, FB3 and FC3, respectively, $p < 0.05$), FB3 rats were no different from controls (Fig. 5A).

Profile analysis for FB1 and FC1 rats showed that the 2 profiles were parallel, $F = 2.60(6, 8)$, $p = 0.11$, and there was no effect for lesion or significant change over time. Univariate ANOVA demonstrated that FB1 rats performed as well as FC1 rats across test days (Fig. 6A).

Heading error. Profile analysis revealed that the profiles for the FB3 and FC3 groups were parallel, $F = 2.62(6, 6)$, p = NS. No effect of lesion was seen, but change over time was found, $F = 4.75(1, 11)$, $p = 0.05$. Although FC3 rats exhibited more day-to-day variability in mean heading error, they were significantly different from FB3 rats only on day 1 (ANOVA; 17.04 ± 7.1 vs. 35.67 ± 5.3 degrees, respectively, $p < 0.05$; Fig. 5B).

For the FB1 and FC1 groups, parallel profiles were obtained, $F = 2.60(6, 8)$, p = NS. No lesion differences or significant change over time was observed and no significant differences in heading error were obtained on any day between these 2 groups (Fig. 6B).

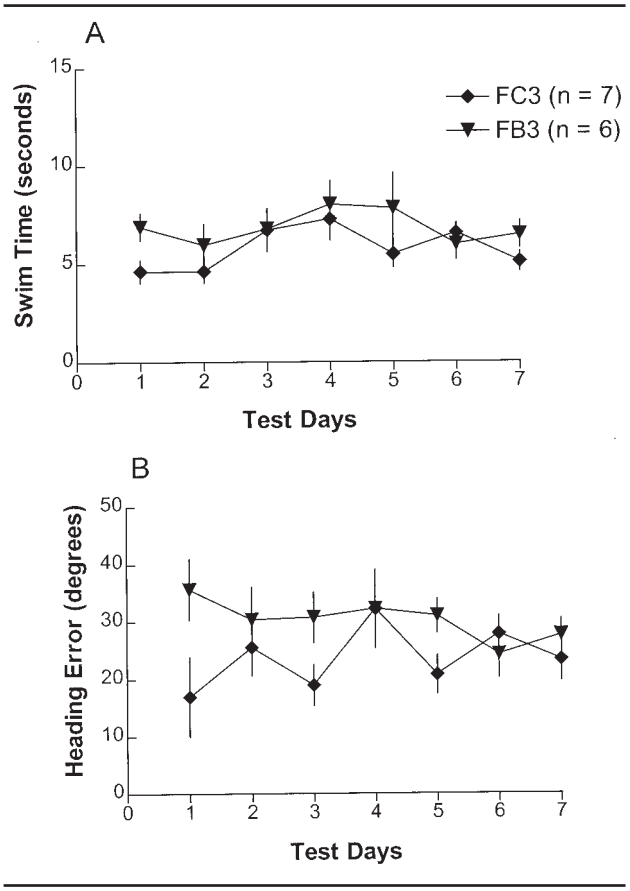

Figure 5. A comparison of mean swim times (A) and directional heading errors (B) for familiar control (FC3) and familiar rats with hippocampal lesions (FB3) initially receiving the distracter on day 3. Means ± SEM for sample size indicated. With the exception of day 1, no differences were seen between the 2 groups.

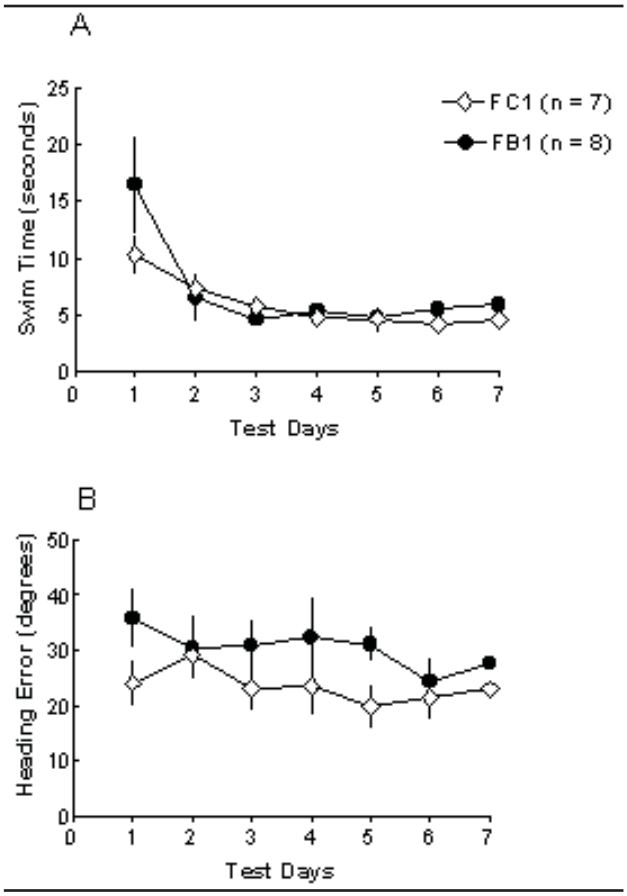

Figure 6. Mean swim times (A) and directional heading errors (B) for familiar control (FC1) rats and familiar rats with hippocampal lesions (FB1) first exposed to the distracter on day 1. Means ± SEM for sample size indicated. FB1 rats performed as well as FC1 rats across test days on both dependent measures.

Responses to the Cue Task by Rats with Hippocampal Lesions

Swim time. To address the hypothesis that familiar rats with hippocampal lesions will locate the goal using a cue faster than unfamiliar rats with hippocampal damage, comparisons were made between the FB3 and UB3 groups. The profiles of the 2 groups were parallel, $F = 0.42(6, 7)$, p = NS (Fig. 3A). Lesion differences were found, suggesting that the effect of familiarity helped FB3 rats behave as if they were not lesioned, $F = 4.54(6, 7)$, $p = 0.03$. No significant change across time was observed. Univariate ANOVA demonstrated that UB3 rats took significantly longer to locate the platform on day 1 than FB rats (21.02 ± 5.6 vs. 6.91 ± 0.7 s, respectively, $p < 0.05$). No significant differences were found between the 2 groups after day 1.

The effect of familiarity with the cue was also examined by comparing FB1 rats and UB3 rats. In this case, not only did the FB1 group have preoperative familiarity with the cue task, but it also had 2 days of exposure to the distracter as compared to the UB3 group. The profiles of the 2 groups were parallel, $F = 0.74(6, 9)$, p = NS. There was a significant effect of lesion ($p < 0.02$), probably from the effect of familiarity. No significant change over time was observed. Univariate ANOVA demonstrated that UB3 rats were

significantly slower than FB1 rats on day 3 (10.22 ± 1.4 vs. 4.56 ± 0.3 s, respectively, $p < 0.05$), day 4 (8.66 ± 1.4 s vs. 5.40 ± 0.6 s, $p < 0.05$), and day 5 (6.77 ± 0.6 vs. 4.82 ± 0.5 s, $p < 0.05$; Fig. 3A).

Heading error. Figure 3B shows the heading error for FB3 and UB3 rats. The profiles of the 2 groups were parallel, $F = 0.62(6, 7)$, p = NS, and no effect of lesion or change over time was seen. No significant differences were obtained between the FB3 and UB3 groups on any day using univariate ANOVA.

Profiles for UB3 and FB1 rats were parallel, $F = 0.27(6, 9)$, p = NS. Lesion differences were obtained ($p < 0.04$), and change across time was significant ($p = 0.04$). Univariate ANOVA revealed that UB3 rats had greater heading error than FB1 rats on day 4 (28.31 ± 3.6 vs. 16.97 ± 3.5 degrees, respectively, $p < 0.05$; Fig. 3B).

Specific Distracter Effects

Another way to determine the degree of distraction is to analyze the contact each group made with the distracter. Two types of analysis were done. In the 1st analysis, contact was defined as the number of times the animals in each group actually touched the distracter during the course of swimming. This was done on day 3 when all groups were exposed to the distracter. In the 2nd type of analysis, contact included the number of times each animal swam within a diameter of 20 cm around the distracter. This analysis combined all days the distracter was in the tub.

Figure 7A demonstrates that UC3 rats touched the distracter almost twice as much as any other group on day 3. This was significantly more often than FB1 rats, which had initially experienced the distracter for 2 previous days (4.17 ± 1.0 vs. 1.88 ± 0.03 times, respectively, $p < 0.05$). No significant differences were noted for any other group.

Results of the 2nd analysis are shown in Figure 7B. UC3 rats entered the 20-cm diameter around the distracter significantly more often the FB1 rats (3.23 ± 0.02 vs. 2.34 ± 0.2 times) and FC3 rats (3.23 ± 0.2 vs. 2.37 = 0.2 times, respectively, $p < 0.05$). Although not statistically different from UC3 rats, FC1, FB3, and UB3 rats were closer in performance to FC3 rats (2.57 ± 0.6, 2.60 ± 0.2, and 2.61 ± 0.2, respectively).

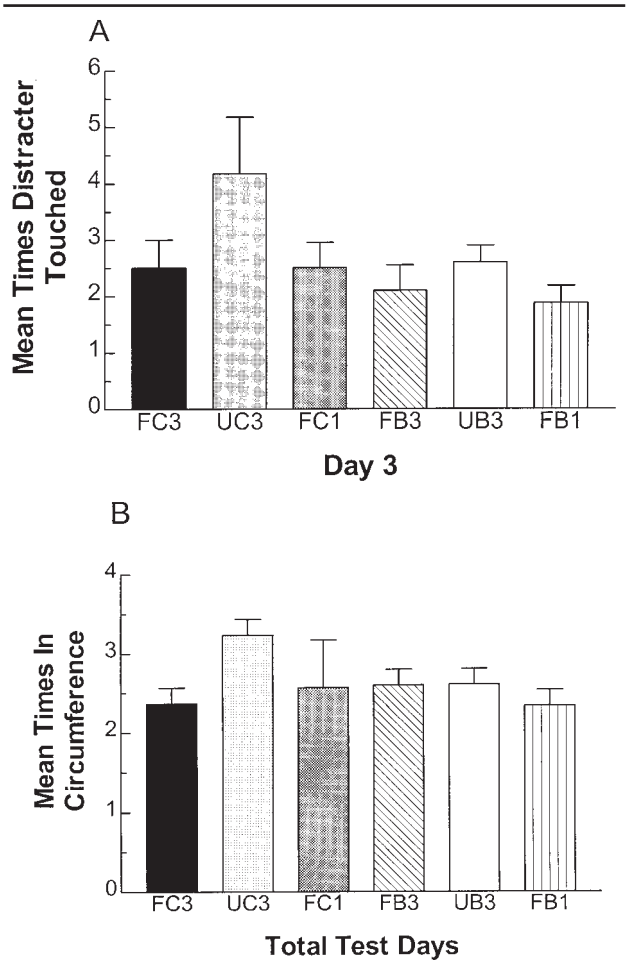

Figure 7. Mean number of times the distracter was touched on day 3 (A) and mean number of times rats entered the 20-cm circumference around the distracter (B). Means ± SEM for sample size indicated.

Discussion

The hypothesis that rats with bilateral hippocampal lesions that were familiar with the cue would perform the cue task as well as control animals in the presence of a distracter was supported in this study. Whether the distracter was introduced on day 1 or day 3, familiar rats with hippocampal lesions performed the cue task as well as control rats as determined by both swim time and heading error. This finding supports those of our initial study (Holden and Therrien 1993), that cue use to identify a specific location is an effective wayfinding strategy for animals with hippocampal impairment

and that familiarity improves the effectiveness of a cue strategy.

In an earlier study, we reported that hippocampal damage as small as 20% was sufficient to disrupt wayfinding in the Morris water test (Holden and Therrien 1993). The average lesion size in the present study was 61% for dorsal hippocampus and 47% for ventral hippocampal lesions. The greatest damage was to the dorsal hippocampus, regardless of overall lesion size. Swanson and others (1987) noted that the ratio of dentate granule cells to pyramidal cells in field CA3 is 10:1 in the dorsal hippocampus, whereas the ratio in the ventral hippocampus is almost 1:1. This suggests that afferents from the dentate gyrus may converge heavily on dorsal CA3 neurons, so that damage to the dorsal hippocampus may disrupt proportionally greater amounts of input than damage to the ventral hippocampus. Additionally, the perforant path, that part of hippocampal circuitry that carries information from the entorhinnal cortex to the dentate gyrus, sends fibers perpendicular to the long axis of the hippocampus (Swanson and others 1987). If so, a small dorsal lesion could disrupt a relatively large amount of input from the perforant path if the damage extended through the hippocampus in a dorsal-ventral orientation, as the lesions in the present study did.

During the course of the experiment, we also found that both familiar and unfamiliar control animals exhibited responses to the distracter. The amount of distraction appears to be related to the amount of familiarity with the cue prior to introduction of the distracter. This statement is based on the observation that FC3 rats, with the most familiarity with the cue prior to distracter exposure, had the weakest responses to the distracter of the 3 control groups. UC3 rats, with only 2 days experience with the cue, showed marked responses to the distracter, whereas performance of the FC1 rats fell between the other 2 groups. Thus, one of the effects of familiarity is to lessen normal responses to distraction during a single cue task. The more familiar the animal is with the cue, the less effect distraction has on performance. The effect of familiarity on distraction in healthy animals appears to be due to a decrease in the exploration of the distracter, as shown by the number of times rats either touched the distracter or swam to within 20 cm of it. Neither of the familiar control groups responded to the distracter as often as unfamiliar controls. However, the knowledge of the cue task in healthy animals does not, by itself, appear to lessen distractibility, as UC3 rats had 8 trials (4 trials per day for 2 days) prior to introduction to the distracter yet still demonstrated a robust interest to the distracter.

Rats with hippocampal lesions familiar with the cue also responded to the distracter, but these responses were not as pronounced as those of control rats because fewer differences were found when consecutive days were compared. As with familiar controls, familiar rats with hippocampal lesions did not actively explore the distracter by touching it or swimming close to it, but the heading error data suggest that the familiar groups with hippocampal damage did not totally ignore the distracter. Rather, they may have noticed the distracter and turned toward it, so that the heading error was large, but then swam off to the cued platform. Because the familiar control and lesion groups were similar in behavior, the lack of exploration appears to be due to the effect of familiarity in the lesioned groups as well as in controls. The extent to which hippocampal damage influenced the familiar lesion groups by diminishing exploratory activity cannot be determined. However, this influence also cannot be completely eliminated as an alternative explanation for the behavior of the lesioned groups because the unfamiliar lesioned group did not respond to the distracter in any apparent way.

A surprising finding was that the amount of distraction experienced by familiar rats with hippocampal lesions was also related to the amount of familiarity with the cue task prior to exposure to the distracter, but in an interesting way. FB3 rats that were exposed to the familiar environment for 2 days prior to the distracter placement did not decrease swim time across test days, whereas FB1 rats exhibited an increased swim time on the 1st day, then decreased swim times below the level of the UB3 animals on subsequent days. These responses are not pronounced, and a larger sample size might clarify these differences. Nonetheless, it does appear that for rats with hippocampal damage, the earlier the exposure to the distracter following lesioning, the quicker and more efficient the adaptation to the change in the environment.

The findings in the present study are in contrast to a study done by Hughey and Koppenaal (1987). They trained rats with dorsal hippocampal lesions to 2 wooden mazes that were identical except that the start

box was switched to the opposite side in the 2nd maze. Tactile and visual cues marked choice points in each maze, and extramaze cues were minimized. Following preoperatively training and lesioning, one group received a baseline trial followed by the switched trials (similar to the FB3 group of the present study), whereas another group was given only the switched box trials (similar to the FB1 group). The baseline switched group made fewer errors than either the switched only group or control rats. However, it is not known whether the arrays of cues in the wooden maze test were equivalent to a cue and distracter in the present study or whether animals respond to multimodal cues differently than to a single mode cue and distracter. Additionally, the intramaze cues were devised to be a cognitive mapping task for control animals, whereas in the present study, every attempt was made to prevent cognitive mapping. It is also difficult to compare the findings of a large-scale spatial task, such as the Morris water test used in the present study, with those of a small-scale task, such as the wooden mazes used by Hughey and Koppenaal. Cognitive mechanisms involved in small-scale spatial tasks may be quite different from those used in large-scale space (Seigal 1981).

In the present study, rats with bilateral hippocampal lesions unfamiliar with the cue demonstrated very little distraction. Means and Douglas (1970) found that rats with dorsal hippocampal aspiration lesions preferred a certain cue in a T-maze and, once exposed to the cue, perseverated to that choice. Perseveration in rats with hippocampal damage is well documented in cognitive mapping tasks (O'Keefe and Nadel 1978; Schenk and Morris 1985), and it follows that perseveration could be manifest in a cue task as well. Coull and Nobre (1998) found that the right posterior parietal cortex is activated in spatial orientation during a small-scale spatial task in humans, which agrees, in part, with O'Keefe and Nadel's (1978) proposal that a cue task is a function of the parietal lobe. However, if the hippocampus is needed to detect changes in a large-scale environment, it may be that loss of hippocampal function leads to some perseverative behavior that makes the use of a cue less effective. Although perseveration may contribute to impairments in learning the cue task after hippocampal damage, it may also prevent unwanted attention to distracting objects in an environment. Studies that introduce distraction earlier in the testing environment for unfamiliar rats, or that use multiple distracters, are warranted.

A troublesome finding in the present study was the extreme variability in heading error among all groups of rats. A weakness in the design of this study may be that random assignment of the distracter placement allowed the cue and distracter to be close during some trials and much farther apart during others. If a rat oriented toward the distracter by turning toward it for one body length, then turned and swam off toward the cue, the directional heading would have been based on the movement toward the distracter. A more accurate measure of directional heading needs to be considered for subsequent studies involving 2 or more environmental cues in the Morris water test.

In this study, we have shown that familiarity with a cue prior to hippocampal damage improves its use as a means of preventing disorientation in the presence of a distracter. The exact mechanism behind this response is not clear, but speculation could be made that familiarity allows an extrahippocampal representation of the cue task to be formed and used after hippocampal damage occurs. If cue representations are stored based on category inclusion, the more familiar the cue, the easier it should be to identify it and use it to prevent disorientation after hippocampal damage. However, it appears that the strongest effect of cue familiarity is obtained when a distracter is encountered soon after hippocampal damage.

The findings of this study have implications for clinical research in people with hippocampal damage. Specifically, human studies that use a cue as a guide for wayfinding in early onset Alzheimer's disease could be used to evaluate the effectiveness of cue familiarity in a real environment after the disease has progressed. For example, Bellin (1990) noted that disoriented humans moved to new environments had difficulty finding toileting facilities even a week after the move. However, in this study no attempt was made by the staff to use a cue to assist the disoriented clients. Bird and others (1995) reported that 4 of 5 patients suffering from dementing illness showed improvements in behavior when environmental cues were used to assist the patients. One patient was able to identify the location of the toilet in his home when the toilet location was marked with a large colored sign. Another patient was able to continue using a cue, a large stop sign, to keep her from inappropriately entering other patient's

rooms when she was moved to a 2nd nursing home. A 3rd patient was able to transfer the knowledge of using a beeper for appropriate toileting from the home environment to a day care facility center. Although the extent of hippocampal damage in the subjects of the Bird and others study was not known, their findings support those of the present study, which suggests that cue use, and especially familiar cue use, has potential as a therapeutic intervention. Further studies need to be done to clarify the role of familiarity when more than one distracter is introduced into an environment and to determine whether there is an optimum time to introduce animals and humans with hippocampal damage to more complex environments.

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The Role of the Pulmonary Afferent Receptors in Producing Hemodynamic Changes during Hyperinflation and Endotracheal Suctioning in an Oleic Acid–Injured Animal Model of Acute Respiratory Failure

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The purpose of this study was to examine the role of the pulmonary afferent receptors in producing hemodynamic changes during hyperinflation and endotracheal suctioning (ETS) in an oleic acid–injured animal model of acute respiratory failure. Previous investigations of hyperinflation as a method to prevent hypoxia-induced sequelae of ETS have demonstrated unrecognized hemodynamic consequences. In this within-subject, repeated-measures study, instrumented, oleic acid–injured dogs had continuous measurements of heart rate (HR), mean aortic blood pressure (MAP), left ventricular pressure (Plv), pulmonary artery pressure (Ppa), right ventricular afterload (Ppa(tm)), right atrial pressure (Pra), and right ventricular filling pressure (Pra(tm)) during hyperinflation and ETS when the vagi were intact and after the pulmonary branches of the vagus nerves had been severed. After severing the vagi, MAP and Plv were decreased and HR and Ppa were increased. With the vagi severed, there was less variation in MAP and Ppa but increased variation in HR. These findings suggest that vagally mediated reflexes from the lungs produce some, but not all, of the hemodynamic effects associated with hyperinflation and ETS. Continued research is necessary to discover a method of hyperoxygenation and suctioning that does not produce potentially harmful hemodynamic effects.

Key words: *Hyperinflation, pulmonary afferents, cardiopulmonary reflexes*

The purpose of this study was to examine the role of the pulmonary afferent receptors in producing hemodynamic changes during hyperinflation and endotracheal suctioning (ETS) in an oleic acid–injured animal model of acute respiratory failure. It was hypothesized that stimulation of the pulmonary afferents by hyperinflation is the mechanism by which some of the cardiovascular effects observed during ETS were

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occurring. If this hypothesis is correct, then these effects should be eliminated or reduced greatly when the pulmonary afferents are severed. An animal model was chosen for this study because it allowed for determination of the mechanisms responsible for the hemodynamic changes associated with hyperinflation when used as a part of endotracheal suctioning. Such a mechanistic study could not be done in human subjects since permanent severing of neural pathways is required. A canine model was chosen because it is a good representation of the pulmonary afferent system (Coleridge and Coleridge 1984).

Although endotracheal suctioning is routinely practiced as an effective means of eliminating pulmonary secretions in patients requiring mechanical ventilation (Baun 1984; Riegel and Forshee 1985), it is not a benign procedure. Reported adverse reactions to ETS include hypoxemia, indicated by a decrease in arterial oxygen tension (PaO_2) and/or a decrease in arterial oxygen saturation (SaO_2) (Fell and Cheney 1971; Adlkofer and Powaser 1978; Skelley and others 1980), atelectasis (Brandstater and Muallem 1969), bronchoconstriction (Woodburne and Powaser 1980), pneumothorax (Loubser and others 1989), introduction of infection (Storm 1980), increased intracranial pressure (Parsons and Shogan 1984; Rudy and others 1986; Durand and others 1989; Rudy and others 1991), trauma to the tracheobronchial tree (Bailey and others 1988; Kleiber and others 1988), cardiac arrhythmias (Shim and others 1969; Winston and others 1987), and death (Marx and others 1968).

Hyperinflation

Because many of the adverse effects are believed to be caused by the development of hypoxia during or after suctioning, various methods of hyperoxygenation and/or hyperinflation prior to, during, and after suctioning have been suggested and evaluated (Fell and Cheney 1971; Naigow and Powaser 1977; Adlkofer and Powaser 1978; Skelley and others 1980; Baun and Flones 1984; Chulay 1988; Chulay and Graeber 1988; Rogge and others 1989). It is now a generally accepted practice to utilize some method to increase the PaO_2 prior to suctioning, between suctioning passes, and after suctioning (Harkin 1975; Kirilloff and Maszkiewicz 1979), but the most effective and safest delivery method has not yet been determined.

The use of hyperinflations prior to, during, and after ETS either with a manual resuscitation bag or the "sigh" mode of a mechanical ventilator produces previously unrecognized, untoward changes in the hemodynamic status of patients. Three studies of the effects of repeated hyperinflation and suction sequences on coronary artery bypass graft patients have demonstrated increases in the mean arterial pressure (MAP) ranging from 9 to 15 mmHg and significant increases in cardiac output (CO) (Preusser and others 1988; Stone and others 1989; Stone, Bell, and others 1991; Stone, Preusser, and others 1991). Another clinical study reported a slight increase in CO and an increase in oxygen consumption during hyperinflation and suction in conscious patients, whereas a decrease in CO was observed in 2 comatose patients (Walsh and others 1989). A study of the effects of intermittent positive pressure ventilation (IPPV) and positive end-expiratory pressure (PEEP) on anesthetized, vagotomized, and Beta-blocked dogs showed an increase in aortic pressure and aortic flow (analogous to CO) during IPPV (Robotham and others 1983).

In contrast, a study of anesthetized dogs undergoing repeated sequences of hyperinflation and suction showed a decrease in MAP after each period of hyperinflation, with a cumulative decrease in MAP of approximately 15 mmHg (Baun and others, unpublished manuscript). When lung hyperinflations were accompanied by an inflation hold of 5 s, the cardiovascular effects were enhanced (Buchanan and Baun 1986). Studies of the effects of IPPV on anesthetized dogs have demonstrated decreases in CO (Morgan and others 1969; Scharf and others 1980), with associated fluctuations in aortic pressure (Scharf and others 1980) or net decreases in blood pressure (Morgan and others 1969). Similar studies of the hemodynamic effects of PEEP on anesthetized dogs revealed decreases in CO (Scharf and others 1977; Cassidy and others 1978), with associated decreases in blood pressure (Cassidy and others 1978), increases in right atrial pressure (Cassidy and others 1978), and diminished left ventricular performance (Scharf and others 1977).

Pulmonary Afferents

One explanation for the hemodynamic changes observed during ETS relates to the role of pulmonary afferent stretch receptors and pulmonary afferent

C-fibers. Pulmonary C-fibers are located near the pulmonary capillaries of the lungs (Coleridge and Coleridge 1984). There is strong evidence that these receptors also influence the cardiovascular system. An experiment in which the lungs of anesthetized dogs were inflated to a pressure of 20 mmHg produced an average 22% decrease in heart rate, a decrease in contractile force, and a decrease in total peripheral resistance. After vagotomy, these effects were virtually absent (Glick and others 1969). These findings strongly suggest that pulmonary afferent C-fibers could play a significant role in the hemodynamic changes observed during hyperinflation of the lungs before, during, and after ETS.

The present study explores the role of the pulmonary afferents during hyperinflation combined with ETS. No previous studies have examined the role of the pulmonary afferents either during ETS or when periods of ETS were combined with hyperinflation. In this study, a comparison is made between prevagotomy and postvagotomy measurements of heart rate, pulmonary artery pressure, aortic pressure, right atrial pressure, and left ventricular pressure during an ETS sequence in which periods of hyperinflation alternate with periods of suction to determine if the hemodynamic changes observed in previous studies can be attributed to stimulation of the pulmonary afferent C-fibers. If the mechanisms behind hyperinflation-induced hemodynamic changes can be explained, a clinically safe method of maintaining oxygenation and ventilation during ETS may be identified.

Methods

Instrumentation of Animals

Five mongrel dogs (25-35 kg) of either sex were used for this study. There are no data to support gender differences in response to the variables of this study. After initial anesthesia was accomplished using Biotal 8 mg/lb (17.6 mg/kg) I.V., the dog was intubated with a cuffed endotracheal tube (9.0 mm internal diameter). Fifteen to 20 min after initial anesthesia, a 2nd dose of Bio-tal equal to approximately one-half the initial dose was given to maintain deep anesthesia while allowing spontaneous respirations. The animal was then connected to the mechanical ventilator and given a loading dose of chloralose (80 mg/kg) for continuing anesthesia. A maintenance dose of chloralose (10 mg/kg) was given every hour, and supplemental doses of pancuronium bromide (0.07 mg/kg) were given, if needed, to suppress spontaneous respirations. The animal was ventilated with a Bennet MA-1 ventilator using a tidal volume of 15 ml/kg and an FIO_2 of 40%. The rate was adjusted to achieve an initial $PaCO_2$ of 35-45 mmHg and pH between 7.35 and 7.45.

A large central venous catheter was placed in the right jugular vein by a cut-down procedure to provide venous access for the administration of fluids and anesthesia. Aortic pressure was measured using a catheter placed into the descending thoracic aorta via the right femoral artery. An Oximetrix Opticath (Abbott Laboratories, Mountain View, CA) 7.5-F pulmonary artery catheter was inserted through the right femoral vein using pressure waveforms to confirm placement. A balloon-tipped catheter (Critikon, 7 F) was placed in the right atrium via the left femoral vein with placement again confirmed by pressure waveform.

A left thoracotomy was performed through the 4th intercostal space by separating and retracting the muscle layers. The pericardial sac was opened and suspended. A tigon catheter was placed into the left ventricle through a small incision at the apex of the heart to measure left ventricular pressure. Two catheters were placed through the open incision into the left hemithorax, 4th to 5th interspace, for measurement of pleural pressure. The lungs were reexpanded using 10 cm H_2O PEEP, the ribs were closed with umbilical tape and plastic wire ties, the muscle layers were sutured with 2-0 chromic, and the skin was closed with 2-0 silk. The chest was evacuated using water seal drainage (Pleur-Evac, Deknatel, Floral Park, NY).

A catheter was connected to the ventilator circuit at the distal end of the endotracheal tube to measure airway pressure. All fluid-filled lines were connected to continuous flush devices and were flushed as needed with heparinized saline. All pressure lines were connected to Statham P23-XL pressure transducers referenced to the mid-thorax and calibrated against a column of mercury. These signals, together with the EKG from needle electrodes on the 4 extremities, were displayed continuously on a calibrated Beckman 8-channel Dynograph or a Gould TA 2000 recorder and on an 8-channel oscilloscope fed into a 16-channel TECMAR Labmaster DMA (Scientific Solutions) in a Zenith microcomputer for analog-to-digital

conversion using Labtech Notebook Software (Laboratory Technologies Corporation, Wilmington, MA), stored on disk, and later analyzed using DADisp Software (DSP, Cambridge, MA).

After prevagotomy data collection (described below), the left thoracotomy incision was reopened, and the pulmonary branches of the vagi were isolated and cut. The incision was closed again as described above. Similarly, a thoracotomy was performed on the right side, the right pulmonary branch of the vagus was cut, the incision was closed, and the chest was evacuated.

Design

A within-subjects, repeated-measures design was utilized for this study in which each subject received the same intervention both with the vagi intact and with the vagi severed, with continuous measurement of the dependent variables. Because of the irreversibility of the vagotomy, the order of the protocols could not be randomized. The independent variable was intact pulmonary vagi versus severed vagi. Dependent variables included heart rate, aortic pressure, pulmonary artery pressure, right atrial pressure, and left ventricular pressure.

Endotracheal Suction

Endotracheal suction was performed through a Bodai-type adapter, which allowed insertion of the suction catheter without removing the subject from the ventilator. Each suction pass consisted of 15 s with the adapter open with 10 s of applied suction (5 s were required for insertion of the catheter and closure of the adapter after suction). Suction was performed using a standard 14-F suction catheter (Pharmaseal) with an external diameter of less than one-half of the internal diameter of the endotracheal tube. The suction catheter was inserted without suction until resistance was felt, withdrawn 2 cm, and then suction was applied continuously for 10 s as the catheter was rotated and withdrawn. Suction was generated by a Gomco Medi-Pump Aspirator (model 130) set to generate a flow rate of 15 L/min as measured by a Fisher-Porter Rotatometer (Warminster, PA).

Lung Inflation

Lung hyperinflations at 135% of tidal volume of 100% O_2 were delivered at the rate of 4 breaths in 20 s by depressing the volume button of a 2nd primed MA-1 ventilator connected through a large 3-way stopcock (Hans Rudolph, Kansas City, MO) so that the system was not opened as the animal was switched from one ventilator to the other. During suctioning, additional breaths at the subject's baseline rate were delivered by the 2nd ventilator. Inflation volumes were measured using an Ohmeda 5420 Electronic Volume Monitor (Ohmeda, Englewood, CO) attached to the 3-way stopcock, and breath-by-breath volumes were displayed continuously on one channel of the Gould TA 2000 recorder.

Procedure

Once the surgical procedures were completed and the animal was stable, oleic acid (0.07 ml/kg) was given I.V. push, and 500 ml of Ringer's lactate was given rapidly via the right jugular vein to induce acute respiratory failure. The animal was monitored until a moderate level of failure was achieved, indicated by a PaO_2 of 50-60 mmHg, usually 1 to 2 h. If appropriate failure was not achieved by the 1st dose of oleic acid, supplemental doses of oleic acid and Ringer's lactate were given until failure was reached. Once the PaO_2 was 50-60 mmHg on an FIO_2 of 40%, the model was considered analogous to a patient with moderate respiratory failure who needs supplemental oxygen to maintain an adequate PaO_2. After the introduction of oleic acid, each animal was brought to a stable baseline as determined by the PaO_2 and $PaCO_2$. pH could not be used for this determination since the oleic acid causes metabolic acidosis.

Each suction sequence consisted of three 15-s periods of open adapter with 10 s of suction preceded and followed by a 20-s hyperinflation period (4 breaths/20 s) after which the animal was returned to the maintenance ventilator (Fig. 1). Continuous recording of all variables occurred at baseline, during each hyperinflation and suction period, at 30-s intervals for the 1st minute after return to the baseline ventilator, and then each minute for 9 min after return to the ventilator. This time frame for the hyperinflation-suction sequence and sampling was determined in previous

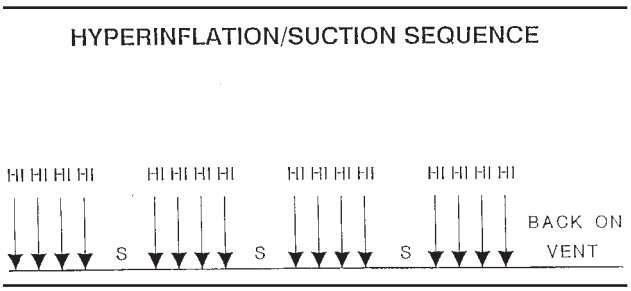

Figure 1. Four hyperinflation breaths (arrows) were given during each of 4 preoxygenation periods prior to and following each of 3 periods of suction. Sampling of all variables continued for 9 min after return to maintenance settings on the ventilator.

studies where it was designed to mimic current clinical practice (Baun and Flones 1984).

Data Analysis

Since all pressure data were recorded continuously, it was necessary to group data according to the events in the hyperinflation/suction sequence. The time periods used for analysis were a baseline period consisting of 4 breaths immediately prior to hyperinflation and suction, 4 hyperinflation periods each consisting of 4 hyperinflations delivered in 20 s, three 15-s periods of suction, 1 breath occurring 30 s after the end of hyperinflation, 1 breath occurring 1 min after the end of hyperinflations, and then during 1 breath every minute for 8 more minutes. For each of these time periods, the data were averaged, and 1 value was reported for the systolic, diastolic, and mean values for aortic pressure, pulmonary artery pressure, and left ventricular pressure, as well as the mean value for the right atrial pressure. Heart rate during each period was determined based on the R-R interval of the EKG.

Because of the small sample size, statistical analyses were not appropriate. Mean values for each data point for each variable were graphed and examined for clinically significant increases or decreases from baseline and for values that fall outside of a clinically acceptable range.

Results

Severing the vagi produced several effects in the hemodynamic variables. After the vagi were severed, the mean aortic pressure (MAP) was generally 10-15

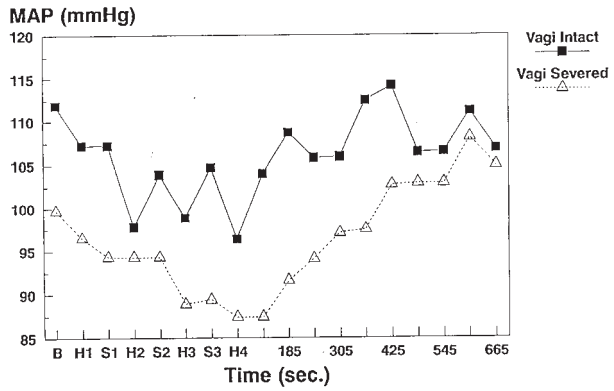

Figure 2. Mean aortic pressure (MAP) for the entire study period when the vagi were intact (solid squares) and after the vagi were severed (open triangles). Each point represents the average value of that period (H = hyperinflation; S = suction). Return to the ventilator at maintenance settings occurred at 125 s, the end of H4.

mmHg lower than when the vagi were intact (Fig. 2). This effect persisted from the baseline observations until nearly 5 min after the hyperinflation/suction protocol. There was an overall decrease in MAP from baseline until the end hyperinflation (H4) regardless of whether the vagi were intact. When the vagi were intact, MAP decreased 5-8 mmHg during each hyperinflation and increased 5-8 mmHg during each period of suction. There was no longer a decrease in MAP during hyperinflation periods H2, H3, and H4 after vagotomy, nor were there increases during suction, but overall there was a 12-mmHg decrease in MAP from baseline to the end of the hyperinflation/suction sequence after which MAP increased to 5 mmHg above baseline 10 min after return to presuction maintenance levels of ventilation.

Examining the systolic left ventricular pressure (Plv) shows that pressures decreased approximately 20 mmHg postvagotomy. As with MAP, there was a gradual decline in systolic Plv from baseline through the end of hyperinflation and suctioning (Fig. 3). This decline was slightly greater postvagotomy (15 mmHg) than it was prevagotomy (10 mmHg). Diastolic Plv recordings were nearly identical under both protocols from baseline through the end of hyperinflation and suctioning, with the postvagotomy values consistently 0.5-1.0 mmHg below the prevagotomy measurements

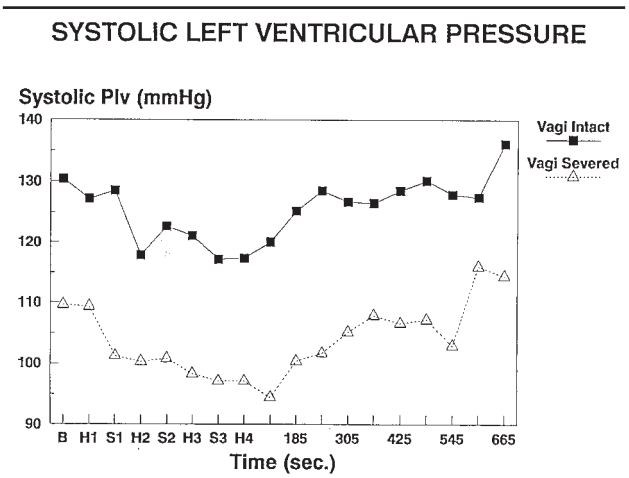

Figure 3. Systolic left ventricular pressure (Systolic Plv) for the entire study period when the vagi were intact (solid squares) and after the vagi were severed (open triangles). Each point represents the average value of that period (H = hyperinflation; S = suction). Return to the ventilator at maintenance settings occurred at 125 s, the end of H4.

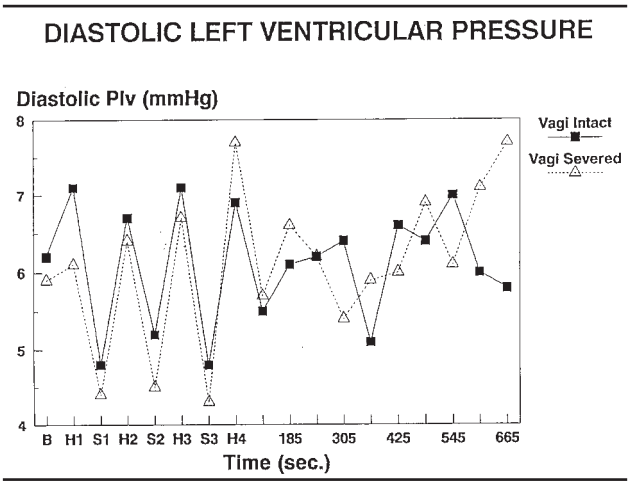

Figure 4. Diastolic left ventricular pressure (Diastolic Plv) for the entire study period when the vagi were intact (solid squares) and after the vagi were severed (open triangles). Each point represents the average value of that period (H = hyperinflation; S = suction). Return to the ventilator at maintenance settings occurred at 125 s, the end of H4.

(Fig. 4). For both protocols, there were increases in diastolic Plv during hyperinflations and decreases during periods of suction.

The mean pulmonary artery pressure (Ppa) was higher postvagotomy by approximately 2 mmHg than prevagotomy (Fig. 5). Again, there was a gradual decrease in pressure from baseline until after the

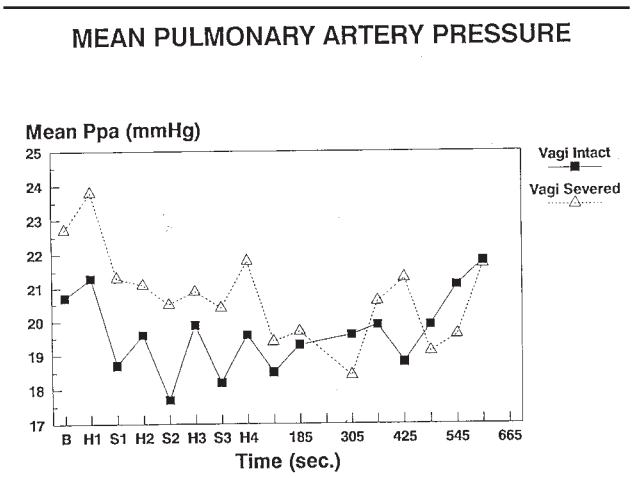

Figure 5. Mean pulmonary artery pressure (MAP) for the entire study period when the vagi were intact (solid squares) and after the vagi were severed (open triangles). Each point represents the average value of that period (H = hyperinflation; S = suction). Return to the ventilator at maintenance settings occurred at 125 s, the end of H4.

hyperinflation/suctioning sequence. Prevagotomy, the Ppa increased during each period of hyperinflation and decreased with each period of suction, with an overall decrease of 2 mmHg by the end of the hyperinflation/suction sequence. The variation between periods of hyperinflation and periods of suction was largely absent postvagotomy, although there was an overall decrease of 3 mmHg from baseline to the end of the hyperinflation/suction sequence.

Right ventricular afterload, as estimated by transmural pulmonary artery pressure (Ppa(tm) [transmural pulmonary artery pressure = mean pulmonary artery pressure – intrathoracic pressure]), was 3 mmHg greater at baseline postvagotomy than at baseline prevagotomy (Fig. 6). Prevagotomy and postvagotomy changes during the hyperinflation/suction sequence were nearly the same. In general, right ventricular afterload decreased during hyperinflation and increased during suction. Values returned to near baseline 10 min after the end of the hyperinflation/suction sequence when the vagi were intact but remained 2-3 mmHg below baseline after the vagi were severed.

Mean heart rate was 12 beats/min faster at baseline postvagotomy. Under both conditions, there was a decrease in heart rate from baseline through the end of S2 or H3 (Fig. 7). Greater variation in heart rate was present postvagotomy, with marked decreases during

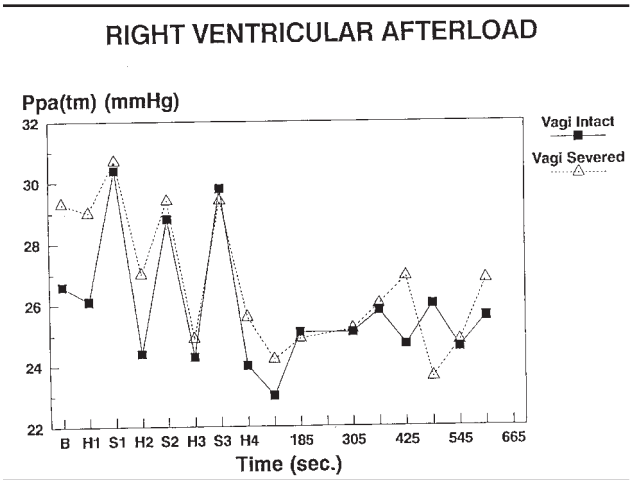

Figure 6. Right ventricular afterload (Ppa(tm)) for the entire study period when the vagi were intact (solid squares) and after the vagi were severed (open triangles). Each point represents the average value of that period (H = hyperinflation; S = suction). Return to the ventilator at maintenance settings occurred at 125 s, the end of H4.

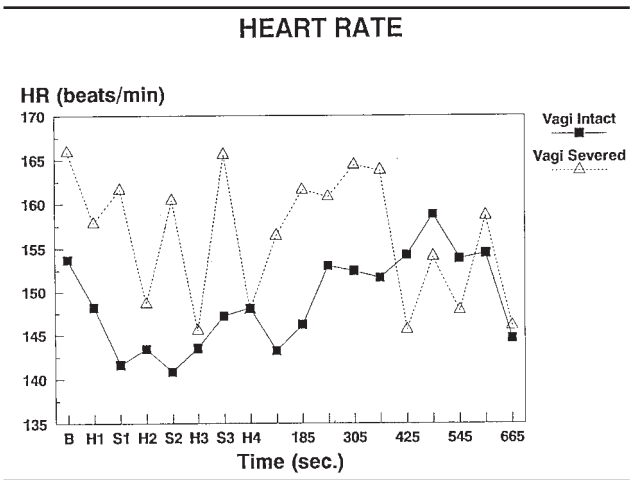

Figure 7. Heart rate (HR) for the entire study period when the vagi were intact (solid squares) and after the vagi were severed (open triangles). Each point represents the average value of that period (H = hyperinflation; S = suction). Return to the ventilator at maintenance settings occurred at 125 s, the end of H4.

periods of hyperinflation and increases during periods of suction.

Prevagotomy measurements of right atrial pressures (Pra) were within 1 mmHg of corresponding postvagotomy values from baseline to the end of the hyperinflation/suction sequence (Fig. 8). Pra increased by approximately 2 mmHg during periods of hyperinflation and decreased by 2 mmHg during periods of suction. When the vagi were intact, Pra decreased immediately after the last hyperinflation and then gradually increased over the 10 min following hyperinflation/suction, ending at 2 mmHg above baseline. After the vagi were severed, Pra gradually decreased during the 10 min after hyperinflation/suctioning to nearly 2 mmHg below baseline.

The right ventricular filling pressure (Pra(tm) [transmural right atrial pressure = right atrial pressure – intrathoracic pressure]) was approximately 2 mmHg greater after the vagi were severed than when the vagi were intact (Fig. 9). Prevagotomy, there were sharp increases in Pra(tm) during S1 and S3 and a smaller increase during S2. The prevagotomy Pra(tm) at the end of hyperinflation/suctioning was nearly the same as at baseline, and then increased a net 2 mmHg over the 10 min following hyperinflation/suctioning. After vagotomy, smaller increases in Pra(tm) were measured during all suction periods. The postvagotomy Pra(tm) at the end of hyperinflation/suctioning was

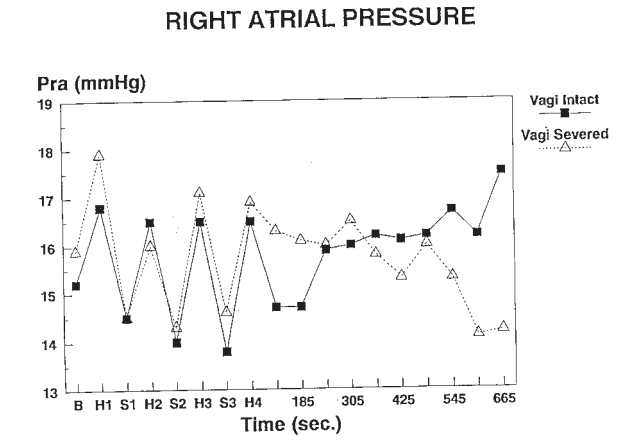

Figure 8. Right atrial pressure (Pra) for the entire study period when the vagi were intact (solid squares) and after the vagi were severed (open triangles). Each point represents the average value of that period (H = hyperinflation; S = suction). Return to the ventilator at maintenance settings occurred at 125 s, the end of H4.

similar to the baseline measurement and then decreased 2 mmHg during the 10 min following the hyperinflation/suctioning sequence.

Discussion

Results of this study demonstrate that severing the pulmonary branches of the vagus nerve results in a

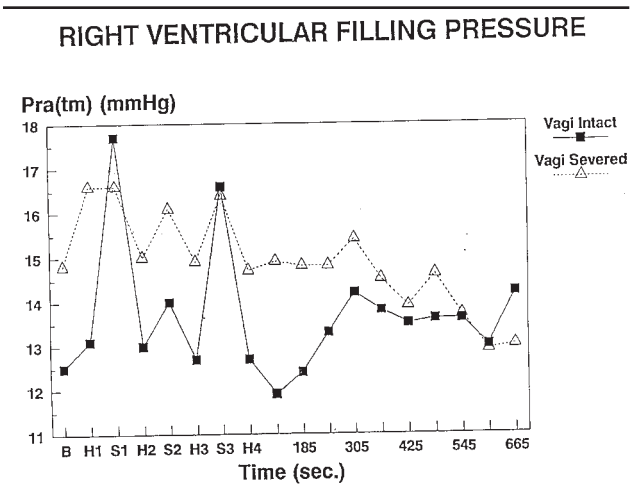

Figure 9. Right ventricular filling pressure (Pra(tm)) for the entire study period when the vagi were intact (solid squares) and after the vagi were severed (open triangles). Each point represents the average value of that period (H = hyperinflation; S = suction). Return to the ventilator at maintenance settings occurred at 125 s, the end of H4.

decrease in MAP and systolic Plv, and an increase in Ppa, heart rate, and right ventricular filling pressure. Severing the vagi also results in decreased variation in MAP, Ppa, and Pra(tm) between hyperinflation periods and suction periods and increased variation in heart rate between periods of hyperinflation and periods of suction. Vagal afferent input from the lower respiratory tract and lungs has been shown to have inhibitory effects on the heart (Coleridge and Coleridge 1984). Much of the investigation of these effects has centered around the role of the pulmonary afferent C-fibers first described by Coleridge and others (1965). These fibers are normally quiet but can be stimulated by hyperinflation of the lungs at 2-3 times tidal volume (Coleridge and Coleridge 1984), which produces bradycardia and a decrease in cardiac contractility (Glick and others 1969; Cassidy and others 1979), a decrease in blood pressure (Salisbury and others 1959; Daly and others 1967; Cassidy and others 1979), a decrease in systemic vascular resistance (Salisbury and others 1959; Daly and others 1967; Daly and Robinson 1968; Glick and others 1969), and an increase in pulmonary artery pressure (Salisbury and others 1959).

The majority of investigations of stimulation of C-fibers were conducted with the dog's chest widely open or with the pulmonary circulation bypassed to eliminate possible mechanical effects of the hyperinflated lungs on the heart and/or great vessels. Also, in many studies, cervical vagotomy was performed, which eliminated or greatly reduced all of the cardiovascular effects produced by hyperinflation. In the present study, only the pulmonary branches of the vagi were severed, which left vagal innervation of the heart and other structures intact, and the animals were studied with closed chests in which the lungs had been re-expanded. This closed-chest model allowed investigation of whether activation of the pulmonary afferents during the hyperinflation/suction sequence is responsible for systemic arterial pressure changes.

The decrease in MAP noted during H2, H3, and H4 prevagotomy was most likely an effect of stimulation of the pulmonary afferent C-fibers (Daly and others 1967; Glick and others 1969; Cassidy and others 1979) as this effect was absent after severing the pulmonary branches of the vagi (Fig. 2). This finding is similar to the results reported by Cassidy and colleagues (1979), who described a preparation of anesthetized dogs designed to eliminate any mechanical restriction to venous return and pulmonary blood flow. In this model, all blood flow and all ventilation were directed to the right lung while the chest was widely open. When the left lung was inflated to 30 cm H_2O for 15 s, there was a 24% decrease in heart rate, a 20% decrease in stroke volume, and a 27% decrease in blood pressure. After transection of the left vasosympathetic trunk at the neck, the transient fall in heart rate, stroke volume, and blood pressure was greatly reduced or eliminated. Similar results were reported by Glick and others (1969), who used cardiopulmonary bypass to bypass the pulmonary circulation. Inflation of the lungs produced negative chronotropic and inotropic effects as well as arterial vasodilation. After bilateral cervical vagotomy, these effects were "markedly lessened." Salisbury and others (1959) also described a decrease in blood pressure induced by hyperinflation that was eliminated by cervical vagotomy.

Since a decrease in MAP by as much as 10 mmHg could be deleterious clinically, it is important to understand the mechanisms by which such decreases are produced. Such knowledge would allow for the development of ETS protocols that do not result in stimulation of the pulmonary afferents.

The role of the pulmonary afferents, however, does not explain the decrease in MAP and systolic Plv from baseline through the end of hyperinflation and suctioning that occurred both when the vagi were intact and when they were severed (Figs. 2 and 3). These decreases may have been produced by vagal receptors located outside of the lungs and therefore operative under both study conditions (Coleridge and Coleridge 1984) or by mechanical effects. It is widely held that lung hyperinflation compresses the great vessels in the chest, impeding venous return and ultimately cardiac output (Morgan and others 1969; Cassidy and others 1978; Smith and others 1982). This explanation does not appear to be satisfactory, however. Even though Pra fluctuated between periods of hyperinflation and periods of suctioning, at no time did it drop to a level low enough to suggest there was inadequate venous return (Fig. 8). This finding could be important clinically. If similar decreases in MAP, and presumably systolic Plv, are observed during sequences in which ETS and hyperinflation are repeated multiple times, then it would be important to limit the number of hyperinflations and suction episodes performed in each sequence.

The transient increases in Ppa during hyperinflation and decreases in Ppa during suctioning that were present when the vagi were intact were diminished after the vagi were severed, suggesting that these variations were vagally mediated (Fig. 5). This finding is consistent with the observations of Salisbury and others (1959), who noted that lung inflations simultaneously produced an increase in pulmonary artery pressure and a decrease in systemic blood pressure.

Past research findings on the activity of the pulmonary afferent C-fibers would predict decreases in heart rate during hyperinflation when the vagi were intact (Coleridge and others 1965; Glick and others 1969; Cassidy and others 1979; Coleridge and Coleridge 1984). Increases in heartrate were noted during the suction periods after the vagi were severed (Fig. 7). It is hypothesized that this is a result of the activity of some of the slowly adapting pulmonary stretch receptors and possibly of the irritant receptors and laryngeal receptors in the upper airways. These receptors promote tachycardia (Daly 1972), but their influence is normally less than the bradycardic effects of the pulmonary C-fibers, and their response is slower. Thus, once the primary effect of the pulmonary C-fibers is eliminated by vagotomy, the tachycardic effect of the slowly adapting stretch receptors becomes apparent several seconds after hyperinflation, that is, during the suction periods and after H4. The general decrease in heart rate present from baseline through the end of hyperinflation/suction under both study conditions may be the result of bronchial irritant airway receptors, which were active both pre- and postvagotomy.

A clinical implication of the decrease in MAP during hyperinflation is that hyperinflation of comatose or heavily sedated patients before, during, or after ETS may not be a safe method of hyperoxygenation. This is especially true of patients who are hypotensive or have marginal blood pressures. Increasing the ventilator rate instead of the tidal volume along with increasing the FIO_2 before, during, and after ETS may produce fewer deleterious hemodynamic changes.

Summary

The findings of this study suggest that the cardiodepressant reflexes associated with pulmonary afferent receptors that have been produced by lung hyperinflation in previous studies are operant during the hyperinflation that is part of the endotracheal suctioning sequence. Other neural and mechanical mechanisms also are involved in producing the multiple hemodynamic effects produced by lung hyperinflation. Although these results cannot be extrapolated directly to humans, the observed effects of hyperinflation both in humans and in canines are similar, and therefore it is reasonable to speculate that the role of pulmonary afferents responsible for these changes are similar.

This study's limitations include its small sample size and the nonrandomized intervention of vagotomy. Future studies could employ vagal cooling or some other form of reversible blockade to control for time effects.

Further research is needed to fully document hyperinflation-induced hemodynamic changes both in humans and in animal models and to explore the multiple competing mechanisms that produce these hemodynamic changes. These efforts will further the goal of producing a clinically safe method for hyperoxygenation and endotracheal suctioning of critically ill patients.

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Negative Life Experiences, Depression, and Immune Function in Abused and Nonabused Women

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*Abuse of women by their intimate partner is a staggering national problem. Abused women have a higher number of medically unexplained somatic symptoms, more functional disability, a lower self-rating of general health, and higher health care utilization when compared to nonabused women. The authors' purpose in this study was to examine differences in occurrences of negative life experiences, level of depression, and T-cell function between abused and nonabused women. The sample consisted of abused women (*n = *12) and nonabused women (*n = *12). Hypotheses tested were (1) abused women will have more negative life experiences than nonabused women, (2) abused women will have higher levels of depression than nonabused women, and (3) abused women will have reduced T-cell function compared to nonabused women. A cross-sectional cohort design was used to compare differences in negative life experiences, levels of depression, and T-cell function. Independent sample* t*-tests were performed comparing the abused versus nonabused women on the dependent measures. Significant differences were found between the groups for negative life experiences (LES;* t = *2.29,* p < *0.05), level of depression (BDI;* t = *3.48,* p < *0.01), and T-cell function (TMR;* t = *–5.62,* p < *0.01). These findings are descriptive and do not establish causal links. However, this is an inquiry into the psychological and biobehavioral responses of women experiencing abuse and their potential health problems. The study shows that abused women reported more negative life experiences, experienced higher levels of depression, and experienced lower T-cell function when compared with nonabused women.*

Key words: *Negative life experiences, depression, T-cell function, abuse*

Abuse of women by their intimate partner is a staggering national problem (Farrell 1996). It is the leading cause of injury to adult females in the United States, exceeding rape, mugging, and auto accidents combined (McLeer and Anwar 1989). Three to four million women are abused by their partner every year (Clarke and others 1997), with an annual cost for health services of approximately $1,633 for each abused woman and a total annual estimated cost of $857.3 million (Meyer 1992). Whether identified or not, abused women are commonly found in every area of health care as well as in work and school settings. Yet, few published studies examine psychological as well as immune function changes in abused women. The purpose of this pilot study was to examine whether

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abused and nonabused women differed on three variables considered to affect psychological and biobehavioral health, specifically, negative life experiences, level of depression, and T-cell function.

Abuse has been likened to a one-way superhighway. One-way because abuse causes health effects, and not the other way around (Walker and Katon 1996), and superhighway because of the complexity, multilanes, and speed at which stress-mediated effects on health of the abused are subjectively assessed (Sobel 1995). Researchers found that two-thirds of the 239 female patients in a gastroenterology clinic reported a prior history of sexual and/or physical abuse (Leserman and others 1996). In comparison with nonabused female patients, the abused have a higher number of medically unexplained somatic symptoms, more functional disability, a lower self-rating of general health, and higher health care utilization. The relationship between abuse and health is exponentially complicated when other mediating factors on health are entered into the analyses (Sobel 1995; Walker and Katon 1996).

Abused women constitute 22% to 35% of women presenting to emergency departments (Loring and Smith 1994; Farrell 1996) and are hospitalized more often than nonabused women (Anderson 1992). In addition to treatment for physical injuries, abused women frequently require health care interventions for substance abuse, eating disorders, suicide attempts, depression, and stress-related illnesses including hypertension, cardiac dysrhythmia, and gastrointestinal disorders (Bergman and Brismar 1991; Swett and others 1991; Rhodes 1993). The long-term health effects of abuse have yet to be fully realized. Health care professionals must develop a greater understanding of the dynamics associated with the abused woman's health problems. Research is necessary to further identify factors contributing to the health risks of these women as well as to develop appropriate intervention strategies.

Seeking protection from abuse (PFA) in court puts the abused at risk for retaliation, further abuse, or even death at the hands of the abuser (Sullivan and others 1992). The most dangerous period for an abused woman is the time when she leaves the abuser (Henderson 1998). In spite of the humiliation of public disclosure of a failed abusive relationship, the abused woman often petitions the court for a PFA only as a last resort to stop the abuse. The time at which the PFA is obtained may be the "open window" phase of the abused individual's experience. She realizes that she is unable to stop the abuse and may be more likely to reach out for help. She may be more open to hearing options available to her to help her escape the abuse, and thus be more receptive to intervention (Constantino and Bricker 1997; Curnow 1997).

The purpose of this study was to examine differences in the incidence of negative life experiences, level of depression, and T-cell function between abused women seeking PFA in court and nonabused women.

Hypotheses

The hypotheses for this study included

1. abused women will have more negative life experiences than nonabused women,
2. abused women will have higher levels of depression than nonabused women, and
3. abused women will have reduced T-cell function compared to nonabused women.

Background

Abuse in this study is defined as any action taken by a male intimate partner with the intent to force or control the emotions or behavior of the female partner, resulting in an imbalance of power. Abuse may consist of such actions as actual or threatened verbal, physical, sexual, psychological, or economic control, including stalking, name calling, destruction of property, or social control through isolation or emotional withdrawal (Constantino and Bricker 1997). The limitation of this definition is in its broadness and the need for self-reporting.

Negative Life Experiences

Life experiences have been found to contribute to changes in levels of stress, depression, and immune function (Baum and others 1993; Suedfield and Pennebaker 1997). Women, who are physically abused by a spouse or a cohabitating partner, are significantly more likely than nonabused women to define their health as fair or poor, to have been diagnosed with a

sexually transmitted disease and other gynecological problems, and to verbalize a need for medical care (Plichta and Abraham 1996). Abuse is therefore seen as an important negative life event in that it has a major impact on the quality of life of the individual.

The Life Experiences Survey (LES) has been utilized to indicate a subject's self-report of life experiences, whether they are seen as positive or negative life experiences. Sarason and colleagues (1978) constructed and utilized the LES in studies to measure life experiences in relationship to stress. The strength of this instrument is in its ability to determine the individual's own response to stress being either negative or positive. In response to specific stressors, a subject rates the stressor on a continuum from "extremely negative" to "extremely positive."

Kiecolt-Glaser and colleagues (1987) found that women who have experienced a negative life event such as separation or divorce for up to 1 year demonstrated lower levels of lymphocyte response to Phytohemagglutinin (PHA). Similar findings showed job loss and unemployment in women were related to significant decrease in lymphocyte response to PHA after 12 months of unemployment (Arnetz and others 1987). Studies on immune function and health indicate that the entire immune system could affect health in acute and chronic stress (Kiecolt-Glaser and others 1996). Zakowski and others (1992) reported that in a group of depressed subjects, even after the termination of the stressor, the stressor's impact on psychological, physiological, and sociobehavioral health may persist for many years. Hypervigilance as reflected in sleep disturbance and use of tobacco and other drugs (TAOD) is a common sociobehavioral characteristic in abused women (Campbell and others 1996). Sleep disturbance in the wake of a stressful event may also signal vulnerability to an adverse stress response (Hall and others 1996).

Depression

Depression is a known psychological dimension of mental health that occurs in abused women. Depression occurs because of the social, emotional, and physical isolation, separation, loss, and the unpredictability that the abuser exacts on the abused (Campbell and Lewandowski 1997). It has long been recognized that individuals who endure abuse often present with depression and that depression as well as suicide have been reported to be common occurrences among abused women (Bergman and Brismar 1991). Stark and Flitcraft (1995, 1996) identified abuse as the single most important cause of female suicides.

Marital discord has been shown to be associated with greater depression and poorer immune function (Kiecolt-Glaser and others 1987). In a sample of 38 separated/divorced women and 38 married women, Kiecolt-Glaser and colleagues (1987) found that in the married cohort, poorer marital quality was associated with greater depression and poorer response on three qualitative measures of immune function (significantly poorer proliferation in response to two mitogens, significantly lower percentages of natural-killer [NK] cells and helper cells, and significantly higher antibody titers to Epstein-Barr virus capsid antigen). Women separated 1 year or less had significantly poorer qualitative and quantitative immune function than their sociodemographically matched married counterparts. And among the separated/divorced cohort, shorter separation periods and greater attachment to the ex-husband were associated with poorer immune function and greater depression.

T-Cell Function (TMR)

T-cell function is one measure of immune function. T cells are lymphocytes, the key cells controlling immune responses. The ability of lymphocytes to proliferate rapidly in the face of an antigen attack is essential to the body's defense. Studies consistently show depression of lymphocyte stimulation in situations of chronic stress, which gives functional assays of lymphocytes strong potential as biological stress markers (Stein and Miller 1991). Decreased mitogen response has been seen in hospitalized patients with depression (Ritchie and Nemeroff 1991; Sternberg and others 1991). Researchers report that many of the physiological sequellae of depression are associated with immunosuppression (Ritchie and Nemeroff 1991; Sternberg and others 1991; Leserman and others 1998), coronary artery changes, atherogenesis, and enhanced sensitivity of the cardiovascular system to catecholamines (Frank and Smith 1990; Benschop and others 1998). All of these changes may be contributing factors in the development of cardiac dysrhythmia and hypertension experienced by abused women. Although studies have

revealed an inverse relationship between depression and T-cell function, few have examined this relationship in abused women (Jackson 1992). There is strong and convincing evidence that moderate to severe depression has immunosuppressive effects (Irwin and others 1990).

Methods

A cross-sectional cohort design was used to compare negative life experiences, level of depression, and T-cell function between abused and nonabused women. The study was reviewed and approved by the Institutional Review Board of the University of Pittsburgh.

Sample

The sample consisted of 12 women who reported being abused by their spouse or intimate partner and 12 nonabused women. The abused sample included women who sought legal representation in court from the local Neighborhood Legal Services pro bono (free) program. At the end of their PFA hearing, subjects were given an Internal Review Board (IRB)-approved flyer containing information about the study and the need for volunteers.

Of 32 clients seeking a PFA in court and who were assigned for representation to the first author (nurse/attorney), 22 showed interest in participating in the study by calling the telephone number found on the flyer. Of the 22 women who volunteered, 15 met inclusion criteria. Of the 15, 12 completed all data collection processes, including blood sampling.

Criteria for inclusion in the cohort of abused women included women, 18 years of age or older, who could speak and understand English, who had been abused by their intimate partner on more than one occasion over the past year, and who were seeking a court order for a final PFA. To qualify for inclusion in the comparison group of nonabused women, the subject must not have experienced abuse in her intimate relationship in the past or the present and reported her intimate relationship as "good" or "excellent."

Exclusion criteria for both groups included the inability to give informed consent; no access to a telephone; hearing impairment, which would preclude their use of a telephone; diagnosis with chronic illness or condition known to influence immune responses, such as diabetes mellitus, heart failure, chronic hepatitis, renal insufficiency, acute infections, severe physical pain, malignancy, steroid treatment, or pregnancy; current psychiatric treatment, psychosis, or cognitive impairment as indicated by confusion or disorientation to time, person, and/or place.

Recruitment of nonabused women was conducted using an IRB-approved flyer announcing the need for volunteers at local civic and religious settings who gave their consent to participate in the study. There were 20 volunteers for the nonabused comparison group, with 15 meeting inclusion criteria. Of the 15, 12 completed all data collection processes including blood sampling.

Mann-Whitney U tests revealed that the 2 groups did not differ significantly with respect to race, age, number of children, alcohol use, medication use, marital status, chronic illness, menstrual status, and use of illicit drugs. The majority of women in both groups did not take any medications on a regular basis, did not abuse alcohol or drugs, had an average of 3 children, and had no chronic illnesses. The women differed on income ($t = 2.39, p < 0.02$), education level ($t = 2.66, p < 0.01$), and tobacco use ($t = 4.28, p < 0.001$), with the abused women having a significantly lower income level and lower educational level. All of the abused women smoked daily (on average, 1 pack per day), whereas only 1 of the nonabused women smoked.

Initiation of contact was made by the participant, who called the number listed on the flyer. The person receiving the call (the first author or the nurse phlebotomist) followed an IRB-approved script in describing the process of becoming a participant in the study. During this telephone conversation, several points were discussed with built-in pauses to allow the caller to process information and to ask questions. Subjects indicating interest in participation were given an appointment for an intake assessment. During the intake appointment, a more detailed description of the study was given and any questions were answered. Subjects were then asked to read the consent form. After all questions were answered, if the subject agreed to participate, she was then asked to sign the consent form. A copy of the signed consent form was given to each subject.

Appointments for the 1st day of testing were then scheduled. Blood sampling was scheduled between 9:00 AM and 11:00 AM to avoid diurnal variations. The subject was provided with instructions to fast after midnight the night prior to the appointment and to avoid smoking and drinking (except water) 1 h before the appointment.

Upon arrival, the subject was seated and given an initial 5-min rest period prior to blood sampling (to measure T-cell function) by a nurse phlebotomist. Subjects were asked to complete the Life Experiences Survey (LES) as a measure of negative life experiences, and the Beck Depression Inventory (BDI) as a measure of level of depression. Surveys were reviewed immediately, prior to the subject's departure, for completeness. Subjects were asked to review and complete the scale if any items were left blank.

Instruments

Life Experiences Survey

The LES was used to obtain a negative life experiences change score. The LES is a 50-item self-report measure that allows respondents to indicate events that they have experienced in the recent past (Sarason and others 1978). The LES contains 47 specific events plus 3 blank spaces that permit subjects to indicate other events that they may have experienced. For purposes of this study, abuse, PFA court hearings, and severity of abuse are recorded in the blank spaces to enable the respondent to rate these occurrences.

The format for the LES requires subjects to rate the desirability and impact of life experiences on a 7-point scale ranging from "extremely negative" (–3) to "extremely positive" (+3). The LES yields a positive life change score, a negative life change score, and a total change score. For purposes of this pilot study, the negative change scores were used to indicate the negative life experience scores for the women. Test-retest reliability coefficients for 5- to 6-week intervals in a sample of college students yielded a positive change score of 0.19 ($n = 34$, $p < 0.001$), 0.53 ($n = 58$, $p < 0.001$), and 0.61 ($n = 12$, $p < 0.05$) at an 8-week interval (Sarason and others 1978). The reliability coefficients for the negative change score were 0.56 ($n = 34$, $p < 0.001$), 0.88 ($n = 58$, $p < 0.001$), and 0.72 ($n = 12$, $p < 0.01$). The coefficients for the total change score were 0.63 ($n = 34$, $p < 0.001$), 0.64 ($n = 58$, $p < 0.001$), and 0.82 ($n = 12$, $p < 0.001$).

In a sample of male and female college students, validity correlations between the LES and the State-Trait Anxiety Inventory ($n = 97$) were 0.03 for positive change scores and state anxiety, 0.46 ($p < 0.001$) for negative change scores and state anxiety, and 0.37 ($p < 0.001$) for total change scores and state anxiety (Spielberger and others 1970). Validity coefficients between the LES and the Beck Depression Inventory (BDI) ($n = 64$) were 0.24 ($p < 0.05$) for negative change (Sarason and others 1978).

Beck Depression Inventory

Level of depression was measured using the BDI. The BDI is a 21-item multiple-choice scale measuring both the presence of and severity of depression (Beck 1996). The summed score for the BDI, which ranges from 0 to 66, was then compared with preestablished levels of depression: minimal (1-13), mild (14-19), moderate (20-28), and severe (29-63).

Studies to establish test-retest reliability revealed a reliability coefficient of 0.90. Item analysis demonstrated a positive correlation between each item of the BDI and the total score ($p < 0.001$) (Beck 1996). The BDI has been shown to have an internal consistency of 0.86 for the items and a Spearman-Brown correlation coefficient of 0.93 for reliability (Stehouwer 1985). Studies in a college population comparing the BDI with psychiatric rating for depth of depression revealed that the concurrent validity of the BDI ranged from 0.65 to 0.77 (Bumberry and others 1978). Internal consistency of the BDI in a study of 42 abused women, calculated with Cronbach's alpha, was 0.80 (Constantino and Bricker 1997).

T-Cell Function

Mitogenic Proliferation: Blood was collected in heparin-treated vacutainer tubes (Becton Dickson) and diluted 1:10 in RPMI 1640 medium supplemented with gentamicin (50 ug/mL), glutamine 2 mM, penicillin (100 U/mL), streptomycin (100 ug/mL), HEPES (10 mM), and 10% (v/v) Fetal Calf Serum (GIBCO, Grand Island, NY). Diluted blood was mixed gently, then added at 100-uL volume per well to triplicate wells of a 96-well plate. PHA was added to triplicate

wells in a 100-uL volume to give final concentrations of 0.5, 2.5, 5, and 10 ug/mL (Sigma). PWM was added to triplicate wells in a 100-uL volume to give final concentrations of 10, 50, 100, and 150 ug/mL (Sigma). RPMI 1640 was added to triplicate wells in place of mitogen to determine spontaneous (background) proliferation. Plates were incubated at 37 °C in 5% CO_2 for 5 days. Wells were pulsed with 1 uCi ^{3}H-thymidine (NEN/Dupont, Boston, MA) for 18-20 h prior to harvesting the culture. Incorporation of ^{3}H-thymidine, measured in cpm, was determined by counting radioactivity using a BetaPlate counter (Wallac, Gaithersburg, MD). CPM of triplicate samples were averaged, and levels of spontaneous proliferation were subtracted prior to determining the Total Mitogen Response (TMR).

Scores of the mitogen responses are given as CPM, both as Net CPM and Total Mitogen Response (TMR) CPM. Net CPM are the experimental test CPM minus the control CPM. The TMR is the sum of the net CPMs at 3 different concentrations, divided by 3. The TMR is used as the final endpoint for analyzing PHA assay results. Using a hypothetical subject ID#041, Lab # 500143, whose blood was drawn on 16 August 1996 as an example, this subject will have 4 values (1 for each of the 3 concentrations in CPMs and 1 TMR). Net CPM 2.5 ug/mL = 19,538, Net CPM 5.0 ug/mL = 21,971, Net CPM 10.0 ug/mL = 25,350, and TMR = 22,286 (Whiteside and others 1990).

The University of Pittsburgh Cancer Institute Immunologic Monitoring and Diagnostic Laboratory performed the T-cell function assays. It sets a normal TMR range between 23,799 and 43,110 and a CPM per concentration as 2.5 ug/mL = 20,823 – 42,086, 5 Fg/mL = 25,062 – 42,551, and 10 ug/mL = 23,347 – 47,727 (Whiteside and others 1990). One laboratory technician received and performed all T-cell function tests.

Results

Simple descriptive statistics are presented in Table 1. Independent sample *t*-tests were performed comparing the abused women with nonabused women on the dependent measures.

Table 1. Means and Standard Deviations of Dependent Measures for Abused and Nonabused Women

Dependent Measure	Group	*N*	Mean	Standard Deviation
Life Experiences Survey (LES)	1	12	17.75	14.45
	2	12	7.50	5.56
Beck Depression Inventory (BDI)	1	12	17.50	8.05
	2	12	8.41	4.10
Total Mitogen Response (TMR)	1	12	16,955	4300
	2	12	27,356	4763

Group 1 = abused women; Group 2 = nonabused women.

Hypothesis 1: Abused women demonstrated more negative life experiences than nonabused women as measured by the LES ($t = 2.29$, $p < 0.05$). Descriptive statistics for the LES revealed that abused women indicated more negative life experiences, with a mean LES negative change score of 17.75 (*SD* = 14.45), compared to nonabused women, who indicated a mean LES negative change score of 7.50 (*SD* = 5.56).

Hypothesis 2: Abused women demonstrated higher levels of depression than nonabused women as measured by the BDI ($t = 3.48$, $p < 0.01$). The abused women experienced various levels of depression, with a mean depression score of 17.50 (*SD* = 8.05), indicating a mild level of depression. The nonabused women had a mean depression score of 8.41 (*SD* = 4.10), indicating a minimal level of depression.

Hypothesis 3: Abused women demonstrated reduced T-cell function compared to nonabused women as measured by TMR ($t = –5.62$, $p < 0.01$). Abused women had lower T-cell function, with a mean of 16,955 (*SD* = 4300), compared to nonabused women, with a mean of 27,356 (*SD* = 4763).

Pearson correlations were used to assess the linear relationships between the dependent measures and are presented in Table 2. Prior to performing these computations, scatter plots were examined for departures from linearity. In all cases, Pearson correlations were found to be appropriate. As can be seen in Table 2, LES negative change scores are significantly related to

Table 2. Correlations of Dependent Measure in Abused (n = 12) and Nonabused (n = 12) Women

Dependent Measure	LES	BDI	TMR
Life Experiences Survey (LES)	1.00		
Beck Depression Inventory (BDI)	0.44	1.00	
Total Mitogen Response (TMR)	–0.33	–0.49	1.00

level of depression (BDI)(r_p = 0.44, $p < 0.05$) and are inversely related to TMR (r_p = –0.33, p = 0.05). As scores increased in self-report of negative events, depression levels increased while T-cell (TMR) response decreased. Level of depression is inversely related to TMR (r_p = –0.49, $p < 0.05$). As level of depression increased, T-cell response decreased.

Discussion

As we enter a new century and a new millennium of cost-containment and cost-benefit in health care, researchers are called to the task of improving health outcomes with cost-effective psychosocial and biobehavioral strategies (Sobel 1995). As previously described, injury to adult females by their intimate partner is a staggering national problem constituting a major cause of all injury to women in the United States. Researchers must establish unambiguous temporal relationships among multiple mediators of health in abuse using valid and reliable measures. The differences in various health concepts between abused and nonabused women should be examined (Walker and Katon 1996). The more physical and psychological energy an abused woman expends in defending herself from her abuser and in coping with the negative events in her life, the less energy she has left for her own survival and functioning (Mahon 1981). Abuse plays a significant role in a woman's feelings of depression, negative self-esteem, and powerlessness, which, in turn, contribute to difficulties in performing daily tasks and responsibilities as parent or worker (Kemp and others 1991).

This comparison group study was designed and conducted to study the negative effects of abuse on health, the needs of the abused, and the high-risk nature of the group. Depression and other psychobehavioral changes are the major sociobehavioral patterns in abused women reported by several researchers (Bergman and Brismar 1991; Campbell and Humphreys 1993; Campbell and others 1996; Leserman and others 1996). Women experiencing marital discord have been found to have lower T-cell function as compared to women not experiencing marital discord (Kiecolt-Glaser and others 1991).

T-cell function, as a component of the immune system, plays an important role in the individual's defense against illness (Kiecolt-Glaser and Glaser 1995; Cohen and Herbert 1996; Leserman and others 1996; Hall and others 1998). It has been proposed that even mild depression may impair the ability of individuals to cope with stress and that this inability to cope may be a reason for succumbing to illness (Coyne and DeLongis 1986). Kiecolt-Glaser and colleagues (1987) suggested that the health consequences of chronic stress may persist beyond the cessation of the stressor if the survivor of the chronic stress is not provided with some form of treatment. In other words, even if the abuse has stopped in response to a PFA court order, the health consequences of abuse may persist if no intervention is provided. To stand idly by in the face of a finding that abused women need some form of help from health care providers would be unconscionable.

We do not know what kind of abuse (verbal, physical, sexual), or what frequency of abuse (i.e., once, twice, three or more times a month) causes the abused woman to report more negative life experiences, increased depression, and lower immune function. This study has shown that women experiencing abuse and seeking PFA reported more negative life experiences and higher levels of depression and were found to have lower T-cell function than nonabused women. It is a first step in the process of identifying the needs of abused women. Research need not be limited to the health care setting. Health care providers practicing in a variety of settings or in nontraditional roles are in a position to make a difference.

Limitations

Results of this feasibility study cannot, at this time, be generalized to other populations of abused and nonabused women. The finding of significant differences in negative life experiences, level of depression, and

T-cell changes must be considered with caution due to the small sample size. Of interest may be what might be different about the women who refused to participate in the study. A significant limitation of this study is the potential conflict of interest when the principal investigator (PI) does research with women who were assigned to her for representation in court. Although she sought their consent to participate in the study after the completion of the PFA hearing, the women may have felt obligated to participate in the study because the representation made by the PI was by pro bono basis. Although coercion may not have taken place, a sense of obligation may have been felt by the participants. In future studies, the PI will not serve as both legal counsel and data collector. Other investigators involved in the study will serve as recruiters for the study.

Although this pilot study showed that there were significant differences between the abused and nonabused groups in levels of depression, the abused women scored in the midrange of mild depression and the nonabused scored in the midrange of minimal. In future studies, positive change scores and the total scores on the LES will also be included in the analysis to examine whether these scores in any way are related to level of depression. One might also question whether a negative change score considering only the events on the 47 primary items might be compared to a negative score that included the 3 abuse items to determine whether there is something unique in negative experiences for abused women versus nonabused women. Phase of the menstrual cycle for premenopausal women may be a variable that can be controlled. The fact that all of the abused women smoked whereas only one of the nonabused women smoked may have been a significant variable in immune function. In future studies, this variable should be controlled.

Conclusion

This pilot study has generated fundamental knowledge about biopsychosocial processes in abused women. The relationships between psychological and behavioral phenomena and possible links to health outcomes in abused women need to be further explored.

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The Effects of Norepinephrine Infusion on the Circulating Lymphocyte Counts of Post–Open Heart Surgery Patients

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In this retrospective study employing chart reviews, 75 open heart surgery patients (OHSPs) were divided into 3 groups of 25 patients. Group 1 received no intravenous (IV) norepinephrine (NE) after surgery. Group 2 and group 3 received a minimum of 0.028 mcg/kg/min of IV NE for 6-24 h and greater than 24 h, respectively. In the 3 groups, preoperative lymphocyte counts were compared to counts obtained on postoperative days 1 and 2. The results showed lower lymphocyte counts on postoperative day 2 in group 3 subjects, who received NE for 24 h or more, compared to subjects of the other groups who received no NE or 6-24 h of NE ($p < 0.05$). There was also evidence that preoperative use of beta-blocking agents significantly affected the change in lymphocyte counts from day 1 to day 2 in both groups receiving NE. Furthermore, postoperative infections were more prevalent in group 3 than the other 2 groups ($p < 0.05$). The lower lymphocyte counts and higher infection rate, however, may be linked to lower postoperative blood pressure and increased number of intensive care unit days in group 3. Further investigation is warranted to elucidate the effects of IV NE administration on the lymphocyte counts of OHSPs and to reduce infections in those receiving NE.

Key words: *Norepinephrine (NE), Lymphocytes, Immune-regulation, and Infection*

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The past few centuries of man's history have been influenced by the belief that the mind can impact one's health, sickness, and recovery (Weil 1995). Only, however, during the later half of the 20th century have the studies scientifically linked the nervous and immune systems (Kiecolt-Glaser and others 1996). It has been discovered that the immune system is linked to the nervous system by sympathetic innervation of the bone marrow, spleen, and lymph nodes (Felten and others 1993). The lymphocyte, itself, has been shown to have receptors for catecholamines released at the nerve synapse (Aarons and others 1980). One receptor, the beta-adrenergic receptor, binds the catecholamine norepinephrine (NE) (Carlson and others 1989).

Recently, investigative efforts have focused on the effects of NE on the immune system and its primary effector cell, the lymphocyte. Studies have determined that NE is a negative regulator of the immune system. That is, NE has been shown to exert its regulatory effects by inhibiting lymphocyte proliferation in the lymph nodes and decreasing migration to the lymph nodes from lymphocyte storage tissues (Madden and others 1993). This is reflected in a lower circulating lymphocyte count as fewer lymphocytes enter the blood stream from the lymph nodes via efferent lymph vessels. Research on both endogenous and exogenous

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NE has supported these findings. Research on the effect of endogenous NE on the lymphocyte in open heart surgery patients (OHSPs) has been conducted (Smiley and Vulliemoz 1992); however, no published research to date has investigated the effects of IV NE administration on lymphocyte counts and infections in OHSPs, who frequently receive this drug.

Purpose and Research Questions

The purpose of this research was to examine the effects of IV NE infusion on immune status in postoperative cardiac surgery patients. The research specifically investigated the effects of postoperative NE administration on the total number of circulating lymphocytes at postoperative day 1 and day 2, compared to preoperative values. The specific research questions were

1. Are the total numbers of circulating lymphocytes in post-OHSPs reduced by NE administration of a mean dose of 0.028 micrograms per kilogram per minute (mcg/kg/min) or more for 6 to 24 h or greater than 24 h, compared to those OHSPs who did not receive NE?
2. What is the effect of preoperative beta-blocking agent administration on the total number of circulating lymphocytes in OHSPs?
3. What is the effect of NE administration on the rate of infection in OHSPs compared to OHSPs who did not receive NE?

Literature Review

Pioneering studies to identify the physiological mechanism by which the mind and body communicate to mediate the immune system were first documented in academic journals in 1968. Initial identification of the pathway was reported in the discovery that nerve fibers of the autonomic nervous system are prevalent in both primary and secondary lymphoid organs. Further research revealed the bone marrow—a primary lymphoid organ responsible for production and differentiation of immune cells—to have significant innervation by sympathetic noradrenergic nerve fibers. High degrees of innervation in the spleen and lymphatic system—secondary lymphoid organs responsible for storage, maturation, and proliferation of immune cells—were also found (Calvo 1968).

Behavioral scientists have spent much effort investigating the effects of stress on immune function in both animals and humans. Studies have shown that stressful situations can negatively affect the immune system, with results ranging from susceptibility to the common cold to increases in tumor growth rate (Sklar and Anisman 1979; Cohen and others 1991). In studies utilizing the rat, a positive correlation was found between the graded level of stress to which the animal was exposed and the suppression of the mitogen stimulation of the immune cells (Keller and others 1981). In human studies, the same immunosuppressive effect was found in individuals after prolonged episodes of bereavement and during academic stress experienced by medical students (Schleifer and others 1983; Malarkey and others 1995).

The results of these and other related psychosomatic studies have been applied to research attempting to alter the immune response in clinical situations in which suppression of the immune system is advantageous. For example, animal studies have used stress induction to decrease the delayed type hypersensitivity reaction to sheep red blood cells (SRBC) and contact sensitivity to the mitogen dinitroflourobenzene (DNFB) (Blecha and others 1982). In clinical studies, a state of stress was purposefully initiated in skin graft patients by modeling stressfully perceived situations. This resulted in successful alteration of the disease process in graft versus host response (Bovbjerg and others 1982).

Further delineation of the nervous-immune systems communication used the knowledge that the nervous system communicates via the release of catecholamines from the nerve synapse to the target cells or organs. Immunization of animals with the immune stimulating antigen or mitogen, SRBC, revealed a decrease in NE levels in the spleen during the immune response. This reduction of NE during immune activity inferred that the presence of NE might in fact inhibit the immune cell function. Further experimental results in this study did, in fact, reveal that low-immune responders are found to have a significantly higher content of NE in the spleen than high responders to the mitogen. The data also revealed a significant increase in splenic weight in high responders, when compared to low responders, from the resultant

increase in immune cell content of the spleen in the presence of decreased NE levels (del Rey and others 1982).

To test whether an increase in lymphocytes is directly related to the decrease in NE release from the nerve synapse, animal studies were conducted in which mice were chemically sympathectomized. That is, the neurotoxic drug 6-hydroxydopamine (6-OHDA) was administered systemically to mice. The noradrenergic nerve terminals were selectively destroyed by this drug, thereby causing depletion in the NE and other catecholamines released to the organs or cells. When the drug-induced sympathectomized mice were compared to a saline-injected control group, the NE content of the lymphoid organs in the sympathectomized group was less than 10% of the control group. The in vivo lymphocyte proliferation of the control and experimental groups was measured by the uptake of injected [^{125}I] deoxyuridine (IUDR) into the DNA of dividing cells. Upon harvest of the animals, the sympathectomized mice exhibited a significant increase in the number of new lymphocytes, measured by radioactivity of the isotope in the spleen, bone marrow, and lymph nodes, suggesting increased cell proliferation in the absence of NE. The inguinal and axillary lymph nodes of the sympathectomized mice displayed an even larger significance of IUDR uptake, as much as 100 times greater than controls, suggesting increased cell migration to these lymph nodes in the absence of NE. This study substantiated the theory that NE exerts a negative regulatory effect on this element of the immune system (Madden and others 1993).

The ability of NE to affect the lymphoid system was further investigated in research on the lymphocyte itself. Researchers identified the beta-adrenergic receptor (beta-receptor) that corresponds to the noradrenergic catecholamines on the surface of the lymphocyte by using a beta-receptor antagonist, which binds to the receptor sites. Different numbers of these beta-receptors were found on the various subpopulations of the human lymphocytes (Krawietz and others 1982). These studies suggested that NE might exert stronger control on specific populations of lymphocytes.

Having established a link between NE and the lymphocyte via the beta-receptor, further research was conducted that focused on the relationship of NE to the immune reaction. NE's actual effect on the lymphocyte was investigated through research involving lymphohematopoiesis, in vivo production, and maturation of the lymphocyte. Animal experiments involving the bone marrow, the site of lymphohematopoiesis, revealed that intraosseous addition of NE to mice resulted in a dose-dependent decrease in the proliferation and differentiation of leukocytes. The decrease in lymphocyte proliferation was evident using as low as 10^{-8} Molar concentrations of NE and resulted in almost a complete inhibition of lymphocyte differentiation and proliferation at concentrations of 10^{-4} molar. This research further supported a regulatory effect of NE on immune cells (Maestroni and Conti 1993).

The human model for examining the effects of elevated levels of NE on the immune system for an extended period of time has been the congestive heart failure (CHF) patient. CHF patients have been documented as having elevated levels of circulating catecholamines due to the compensatory action of the autonomic nervous system for controlling blood pressure. These patients have also been documented as immune compromised, suggesting a correlation between these clinical manifestations. To further examine this possible link, the rat animal model was studied by administering NE and other catecholamines for a period of 4 weeks to an experimental group of rats. The results of the NE infusion showed a dose-dependent decrease in proliferation of T cells and an overall decrease in the total number of T cells. However, exposure of B cells to a high level of NE for a prolonged period of time actually led to an increase in B cell proliferation and count. This suggested that consistently high levels of NE might lead to immune compromise in CHF patients by down-regulation of the beta-receptor leading to a block in the negative regulatory mechanisms of the cell. This may have caused an inhibition in differentiation of antibodies to specific mitogens due to premature proliferation of B cells.

The OHSP was also used as a model to investigate the effects of exposure to prolonged bursts of NE. The OHSP is exposed to extreme physiological stress while on cardiopulmonary bypass and under the surgical procedure itself. This physiological stress, along with anaesthetic and vasolytic drug use, can result in a very high level of circulating catecholamine levels. Studies to test the effects of this intraoperative stress on the OHSP were conducted. Blood samples from

OHSPs were drawn before and after surgery. The samples were then stimulated with a beta-adrenergic agonist, isoproteronol. The cAMP production of the cells was measured to indicate beta-receptor binding and activation. The postsurgical blood when compared to the presurgical blood showed significantly less cAMP when stimulated with isoproteronol, reflecting a decrease in the binding of the receptor. The researchers in this study hypothesized this to be a desensitization of the beta-receptors on the lymphocyte surface (Smiley and Vulliemoz 1992).

The number of existing beta-receptors on the lymphocyte has been shown in research to be subject to up-regulation as well. Clinical investigation of the administration of beta-adrenergic blocking agents revealed that prolonged exposure of the cells to the beta-blocking agent, propanolol, caused an increase in beta-adrenergic receptors of human lymphocytes. The up-regulation of the beta-receptors was measured at a 43% increase after only 5 days of the drug propanolol. Withdrawal of the drug resulted in a return of the beta-receptor count to normal within 24 h (Aarons and others 1980). However, it was hypothesized that patients with beta-receptor up-regulation from chronic beta-blockade may be highly susceptible to beta-receptor desensitization if the beta-blocking agent is removed and the patient experiences high levels of catecholamine levels from cardiac surgical stress (Smiley and Vulliemoz 1992).

The exact mechanisms by which the nervous and immune systems interact are very complex in nature. The detailed research in this area has identified many of the probable signaling methods and pathways. An overwhelming amount of literature on the subject has supported the hypothesis that NE is one of its major messengers and the beta-receptor on the lymphocyte is the target for NE's regulatory mechanism. This regulatory mechanism of the lymphocyte by NE appears to be a decrease in migration of lymphocytes to the lymph nodes as well as a decrease in proliferation of these lymphocytes. The result of this negative regulatory mechanism is a decrease in immune activity by lymphocytes in the presence of NE.

Clinical studies involving CHF patients have shown that chronic elevation of endogenous NE may lead to down-regulation of the beta-receptors on the lymphocytes, causing a decrease in immune regulation by NE. Prolonged bursts of both endogenous and exogenous NE have also been show to affect NE regulation of the lymphocyte by beta-receptor desensitization. Furthermore, beta-receptor blockade resulting from the use of beta-blocking drugs can also influence the beta-receptors on the lymphocyte by causing an up-regulation of these receptors. It is hypothesized that once the beta-blocking agent is removed, the regulation of the lymphocytes by NE is enhanced due to a greater number of receptor sites. A brief summary of the possible relationship between the NE aspect of the nervous system and lymphocyte portion of the immune system is illustrated in Figure 1.

Methods

A retrospective chart review was performed in 3 groups of OHSPs:

- Group 1—records from patients who received no IV NE following complications of the surgical procedure; identified as the NO NE group (No NE).
- Group 2—records from patients who received 6 to 24 h of a minimal IV NE dose of 0.028 mcg/kg/min following complications of the surgical procedure; identified as the less than 24-h NE group (<24hr NE).
- Group 3—records from patients who received more than 24 h of a minimal IV NE dose of 0.028 mcg/kg/min following complications of the surgical procedure; identified as the greater than 24-h NE group (>24hr NE).

To ensure that the groups were comparable, the APACHE II, a morbidity and mortality tool (Knaus and others 1985) was used to ensure group similarity in physiological parameters/status immediately prior to surgery. Each group was chosen from a sample of patients with an APACHE II score of 17 or less. All patients were admitted to a cardiovascular ICU immediately postoperative for recovery and remained in the ICU for at least 24 h. Further selection criteria as defined in Table 1 ensured similarities both within and among the 3 groups.

The sample population was selected from an OHSP population of a Level I hospital in southern Arizona. The selected hospital was a regional medical center

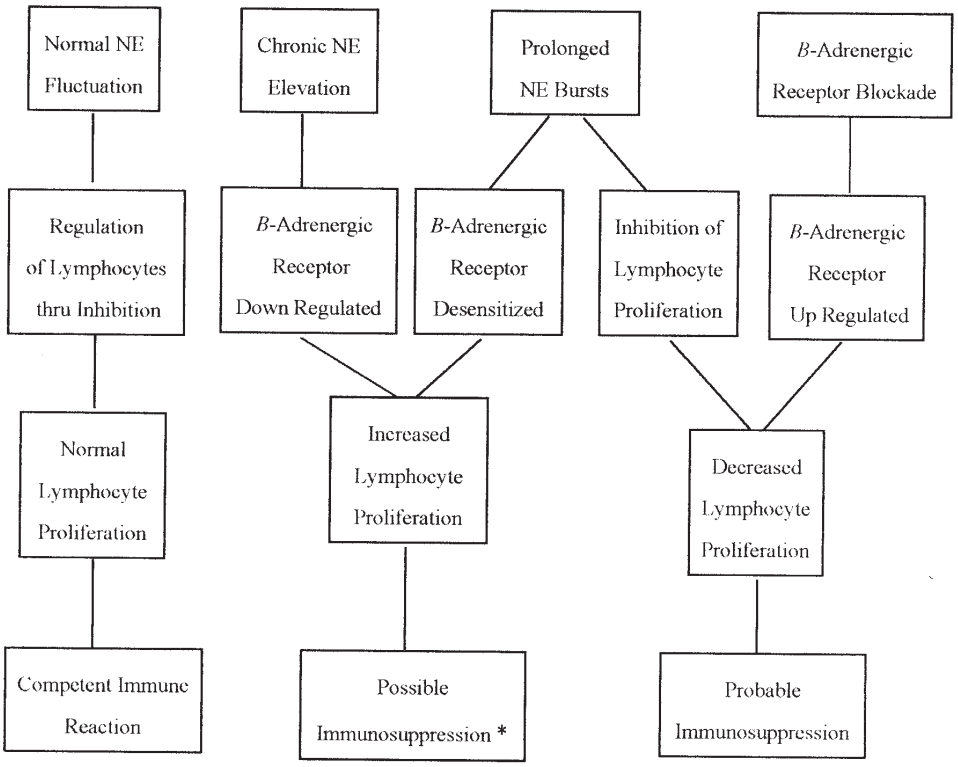

Figure 1. Summary of Possible Nervous-Immune System Interaction Patterns.
*From premature lymphocyte proliferation.

responsible for the performance of 35% of cardiac surgeries in the area. The total sample of records from 75 subjects was divided into the 3 groups based on the amount of NE received after surgery. Each group contained 25 individuals, as determined by a sample size power analysis, to produce a power level of 0.82.

For patient selection, a computer search of 1996/1997 hospital records was completed using the appropriate diagnostic-related group (DRG) codes for OHSPs. The computer records revealed a population of 987 patients between the ages of 35 and 75 who had elective open heart surgery and no pharmacy charge for the drug NE. A computer program designed for random sampling randomly selected 45 patients from this group. There were 28 complete charts available for review from the sample. None had an APACHE II score greater than 17 or received NE therapy during his or her hospitalization. Manual random selection of 25 patients produced the sample population for group 1 (No NE).

A further computer search of the 1996/1997 hospital records, which matched the appropriate DRG codes

Table 1. Criteria for Selection of Sample

Variable	Observations and Treatments
Cardiac diagnoses (1 or more of the following diagnosis)	Acute myocardial infarction Unrelieved angina pectoralis Valvular insufficiency Valvular stenosis Atrial septal defect
Surgical procedure (1 or more of the following non-emergency surgeries)	Cardiac arterial bypass graft Valvular replacement/repair
Surgical stabilization (All of the following)	Use of general anesthesia Endotracheal intubation Cardiopulmonary bypass for at least 1 h
Postsurgical drug therapy (1 of the following post-operative day 1 or 2)	Use of IV NE for 6-24 h Use of IV NE for > 24 h No use of IV NE after surgery
Preoperative Glascow Coma Score	15
APACHE II Score	Less than or equal to 17

Table 2. Description of the Sample by Group

	Group 1 (No NE)	Group 2 (<24h NE)	Group 3 (>24h NE)	Significance
Sex				
Female	6[a]	2[a]	9[a]	sign[a]
Male	19	23	18	
Age				
Mean	62.5 ± 8.4	62.6 ± 9.9	64.8 ± 6.8	ns[b]
Range	41 – 75	37 – 75	46 – 76	
APACHE II Score				
Mean	8.8 ± 1.0	9.1 ± 2.0	9.7 ± 1.2	ns[b]
Range	7.0 – 11.0	6.0 – 16.0	7.0 – 12.0	
Preoperative Lymphocytes				
Mean	1890.8 ± 744.8	1962.6 ± 928.7	1744.7 ± 525.6	ns[b]
Range	190.0 – 3649.0	270.0 – 4557.0	720.0 – 3171.0	

NOTE: Results are mean ± *SD* with 25 subjects in each group.
sign[a]: significant (1-way ANOVA: $p < 0.05$).
ns[b]: not significant (2-factor ANOVA with replication: $p > 0.05$).

to a hospital pharmacy charge for NE, revealed 151 patients between the ages of 35 and 75 who had elective open heart surgery and a pharmacy charge for NE. There were 127 complete charts available for review. Of these patients, 44 were eliminated due to procedures other than valvular repair/replacement or coronary artery bypass, death resulting from surgical complications, or lack of documented NE use. Of the remaining 83 patients, 34 met the selection criteria for group 2, 6 to 24 h of NE infusion (<24h NE) and 26 met the criteria for group 3, greater than 24 h of NE infusion (>24h NE). Of these patients, none had an APACHE II score greater than 17. Manual random selection of 25 patients for each group resulted in the sample population for groups 2 and 3. Hence, the final sample consisted of 75 patients with 25 patients in each group.

The chart reviews included data collection from the history and physical, anesthesia record, operatory report, laboratory records, physician's notes, nurse's notes, drug administration records, and nurse's flow sheets. The demographic data collection included age, sex, weight, diagnosis, surgical procedure, time on bypass, mortality, and complications. The research data for analysis included preoperative complete blood count (CBC) with differential, 1st postoperative morning CBC with differential, and 2nd postoperative morning CBC with differential. Further data collection included length of NE infusion, dose of NE infusion, and other beta agonistic drugs, anti-inflammatory drugs, and postoperative infections.

The NE dose was recorded using the standard method of dosage administration of micrograms per minute and adjusting for variations in weight by dividing the recorded dose by the patients' preoperative weight in kilograms. The total lymphocyte counts from the CBC with differential for each time period was calculated by multiplying the total WBC by the percentage of these cells, which were lymphocytes. Statistical analysis was conducted on the data utilizing various methods of analysis of variance (ANOVA) with multiple comparison corrections to meet the 0.95 confidence level of significance. The probability for significance level was set at $p < 0.05$.

Results

Each of the 3 groups in this study, group 1 (No NE), group 2 (<24h NE), and group 3 (>24h NE), contained 25 patients who met the criteria for selection in Table 1. The demographic characteristics, APACHE II scores, and preoperative lymphocyte counts of each group are shown in Table 2. There was no significant difference between age and APACHE II scores between the groups ($p > 0.05$). There was, however, a significant difference in the male to female ratio in group 2 (<24h NE) compared to the other groups ($p > 0.05$).

Table 3. Postoperative Characteristics of the Sample by Group

	Group 1 (No NE)	Group 2 (<24h NE)	Group 3 (>24h NE)	Significance
Surgery				
CABG	22	21	18	ns[b]
MVR	3	2	3	
AVR	2	5	8	
ICU Days				
Mean	1.9 ± 0.5[a]	3.2 ± 2.4[a]	5.6 ± 2.8[a]	sign[a]
Range	1.0 – 4.5	1.0 – 9.0	3.0 – 14.0	
Total Hospital days				
Mean	6.5 ± 2.8[a]	8.2 ± 2.1[a]	12.6 ± 3.8[a]	sign[a]
Range	4.0 – 19.9	6.0 – 15.3	7.0 – 19.1	
Postoperative Antibiotic Therapy (# of Days)				
Mean	2.2 ± 0.5	2.1 ± 0.4	2.4±1.0	ns[b]
Range	2.0 – 4.0	2.0 – 4.0	2.0 – 5.0	

NOTE: Results are mean ± *SD* with 25 subjects in each group. NE = norepinephrine.
sign[a]: significant (2-factor ANOVA with replication: $p < 0.05$).
ns[b]: not significant (1-way ANOVA: $p > 0.05$).

Table 4. Pre- and Postoperative Drug Therapy by Group

	Group 1 (No NE)	Group 2 (<24h NE)	Group 3 (>24h NE)	Significance
NSAID[a]				
Preoperative	8	11	12	ns[a]
Postoperative	25	24	24	
Steroids				
Preoperative	1	3	1	ns[a]
Postoperative	0	0	0	
β-blocking agent				
Preoperative	7	9	10	ns[a]
Postoperative	3	3	1	
Norepinephrine				
Postoperative	0	25	25	n/a

NOTE: Numbers are number of patients receiving drug. n/a = not applicable.
ns[a]: not significant (1-way ANOVA: p value > 0.05).
NSAID = Nonsteroidal anti-inflammatory drug.

The postoperative characteristics of each group are shown in Table 3. There was a significant difference in the total number of ICU days among all 3 groups and a significant difference in total number of hospital days for group 3 (>24h NE) when compared to the other 2 groups ($p > 0.05$). Data collection also included the preoperative and postoperative drug therapy for each patient. The pertinent drugs for this study with the number of patients receiving each drug per group are identified in Table 4. The basis for treatment with NE for patients in group 2 and group 3 was intraoperative or immediate postoperative hypotension that was unresponsive to initial documented use of dopamine.

There was an overall decrease in the mean circulating lymphocyte count in all 3 groups, with no significant difference between groups on postoperative day 1. There was a significant difference in the mean circulating lymphocyte count on postoperative day 2 for group 3 when compared to groups 1 and 2 (see Fig. 2). The percentage of increase in the mean circulating lymphocyte count from day 1 to day 2 was also

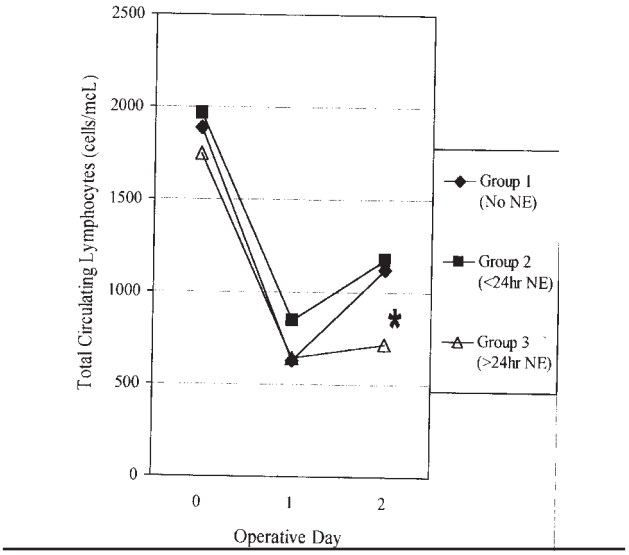

Figure 2. Mean Circulating Lymphocyte Count by Group. Twenty-five subjects in each group. (See Table 5 for ranges.) *Significant difference (2-factor ANOVA with replication: $p < 0.05$).

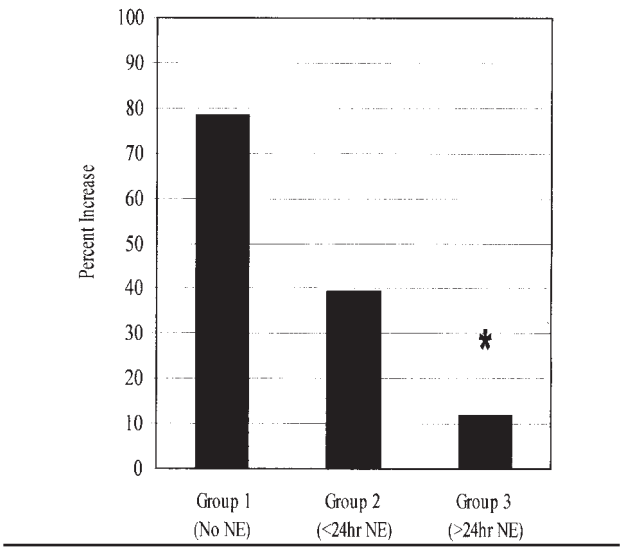

Figure 3. Mean Percentage Increase in Circulating Lymphocytes from Postoperative Day 1 to Day 2 by Group. Twenty-five subjects in each group. Ranges: group 1 (51%-95%), group 2 (11%-59%), group 3 (–12%-28%).

Table 5. Mean Circulating Lymphocyte Counts by Group

	Group 1 (No NE)	Group 2 (<24h NE)	Group 3 (>24h NE)	Significance
Preoperative (cells/mcL)				
Mean	1890.8 ± 744.8[b]	1962.6 ± 928.7[b]	1744.7 ± 525.6[b]	ns[b]
Range	190.0-3649.0	270.0-4557.0	720.0-3171.0	
Day 1 (cells/mcL)				
Mean	631.7 ± 258.9[b]	844.9 ± 321.5[b]	642.0 ± 214.7[b]	ns[b]
Range	65-1001.0	0.0-1450.0	0.0-1161.0	
Day 2 (cells/mcL)				
Mean	1126.1 ± 328.5	1178.4 ± 341.0	718.8 ± 198.9[a]	sign[a]
Range	328.0-2550.0	0.0-2770.0	0.0-1161.0	

NOTE: Results are mean ± *SD* with 25 subjects in each group. NE = norepinephrine.
sign[a]: significant (2-factor ANOVA with replication: $p > 0.05$).
ns[b]: not significant (2-factor ANOVA with replication: $p > 0.05$).

significantly different in groups 1 and 2 when compared to group 3 (see Fig. 3).

There was no significant difference between preoperative and postoperative lymphocyte counts within the groups when comparing those who received preoperative beta-blocking agents to those who did not. When taking into consideration the effects of NE on both migration and proliferation, further investigation of the actual change in lymphocyte counts was conducted. Although the actual mean lymphocyte counts revealed no significant difference between beta-blocking agent and non-beta-blocking agent recipients ($p > 0.05$), there was a significant difference in the percentage change in the lymphocyte counts both within and between groups from day 1 to day 2 (see Table 5; $p > 0.05$). Those who did not receive beta-blocking agents preoperatively had a significantly greater percentage increase in the mean lymphocyte counts in both groups receiving NE. The beta-blocking agent recipients in group 2 (<24 h NE) had a minimal percentage increase in the mean lymphocyte count, whereas group 3 (>24 h NE) had an actual decrease in the mean lymphocyte count. There was also a

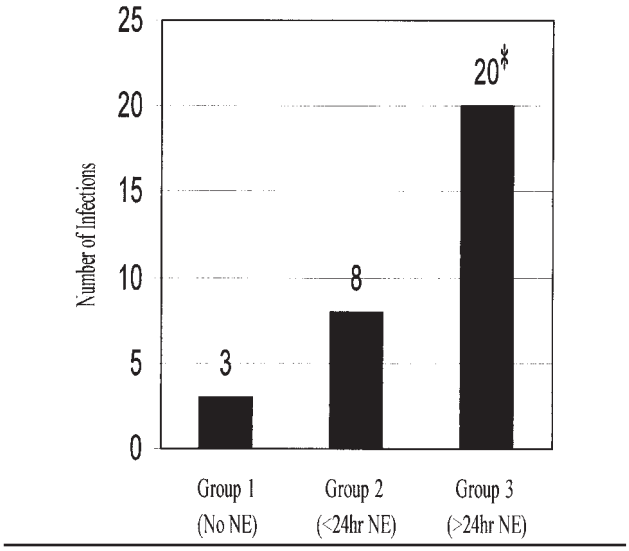

Figure 4. Number of Postoperative Infections by Group.
*Significant difference (1-way ANOVA: $p < 0.05$).

significant difference between both NE groups and the No NE group ($p > 0.05$) when comparing this change.

Because there was no significant variation of NSAID, steroid, or postoperative beta-blocking agent use among the groups (1-way ANOVA, $p > 0.05$), these data were not presented. Additionally, there was no significant difference within or between the groups in lymphocyte counts when comparing the preoperative diagnosis of CHF to other diagnoses (1-way ANOVA, $p > 0.05$); therefore, these data were also not presented.

There was a significant difference (1-way ANOVA, $p > 0.05$) in documented postoperative infection between group 3 (>24h NE) and the other 2 groups (see Fig. 4). Postoperative infection was identified by a positive laboratory culture for bacterial presence within 5 days of surgery and documentation by the physician in the progress notes. The time frame for infection was limited to allow for the earlier discharge time of the No NE group and other infectious exposure besides surgery.

Discussion

The findings of this study are consistent with the hypothesis that NE infusion can alter the circulating lymphocyte counts of OHSPs. The results suggest beta-blocking drugs given preoperatively may affect both endogenous and exogenous regulation of lymphocytes. Also, these findings are consistent with increased infections in this population with NE infusion. However, the findings related to NE and lymphocyte counts causing a direct effect on increased infection rates in group 3 are not conclusive due to differences in linked variables in group 3 compared to the other 2 groups, namely, sex, low blood pressure complications, and ICU days.

The extraneous variables that may have affected the results were attempted to be controlled by following the selection criteria in Table 1 and prescreening all subjects with the APACHE II tool. However, a number of variables were not able to be addressed using the chart review format. There are some identified differences in the sample characteristics of each group that may have affected some results. The fewer number of females in group 2 (<24h NE) may have affected the mean lymphocyte counts in this group compared to the other 2 groups. Although there is no difference in the documented standard lymphocyte counts between men and women, there may be some differences in the immune reaction process between the sexes, because hormones affect production, storage, and release of lymphocytes. Additionally, hypotension may also have been a risk factor creating increased levels of endogenous NE and cytokines, which may have affected lymphocyte counts in groups 2 and 3.

The postoperative characteristics revealed a significant difference in both total ICU days and total hospital days. Because all documented infections were recorded by the 5th postoperative day, it is doubtful that the increased number of total hospital days contributed to a higher infection rate of group 3 (>24h NE). However, an increased number of ICU days has been linked to higher infection rates (Nicholls 1997) and may have had some influence within this group.

The mean circulating lymphocyte count of all 3 groups was lower on both postoperative day 1 and day 2 than the preoperative value. This could be related to the postoperative inflammation process causing a migration of immune cells to the surgical sites and surrounding tissue (Quinn and Shannon 1975) as well as increased cortisol levels from surgical stress causing a reduction in lymphocyte production (Smiley and Vulliemoz 1992). The mean postoperative day 2 lymphocyte count was relatively higher in all 3 groups when compared to day 1 (see Fig. 2). This relative increase in

Table 6. Mean Percentage Change in Circulating Lymphocytes from Postoperative Day 1 to Day 2 with and without β-Blocking Agent by Group

	Group 1 (No NE)	Group 2 (<24h NE)	Group 3 (>24h NE)
No β-blocking Agent	45 ± 21[a] (n = 18)	40 ± 17[a] (n = 16)	37 ± 17[a] (n = 15)
β-blocking Agent	83 ± 39[ab] (n = 7)	11 ± 3[ab] (n = 9)	–16 ± 4[ab] (n = 10)

NOTE: Results are mean percentage ± *SD*. n = number of subjects on drug. NE = norepinephrine.
a. significant difference within groups (2-factor ANOVA without replication: $p < 0.05$).
b. significant difference between groups (2-factor ANOVA without replication: $p < 0.05$).

circulating lymphocytes may be explained by 2 distinct events:

An overall increase in the total number of lymphocytes due to proliferation, and
An influx of lymphocytes back into circulation from the tissue via lymph vessels after migration to the lymph nodes (Quinn and Shannon 1975)

The mean percentage increase in circulating lymphocytes from day 1 to day 2 was lower in both of the NE groups than the No NE group. Group 3 (>24h NE) had a significantly lower count than either of the other 2 groups (see Table 6). Because NE negatively regulates both proliferation and migration of lymphocytes, the administration of exogenous NE may have contributed to a decrease in these activities in both NE groups. The significant difference in group 3 (>24h NE) could be due to the significantly higher dosage of NE used in this group along with the increased time that the drug was used.

When comparing the patients receiving beta-blocking agents preoperatively to those who did not, the trend in all 3 groups can be seen in Table 5. All patients receiving beta-blocking agents preoperatively had a significant difference in the percentage change in circulating lymphocytes from day 1 to day 2 within the group, when compared to the non-beta-blocking agent patients. This smaller percentage change was mainly caused by a higher mean circulating lymphocyte count on day 1 in the beta-blocking agent patients of each group. This resulted in a relatively smaller increase in the mean circulating lymphocyte count of beta-blocking agent recipients in group 1 and 2 on day 2 and an actual decrease in the mean lymphocyte count of beta-blocking agent recipients in group 3 on day 2.

The significantly higher rate of infection among patients in group 3 (>24h NE) (see Fig. 4) may have a positive correlation to the lower lymphocyte counts in these patients. All of the patients in group 3 with a documented postoperative infection, n = 20, received only 2 days of antibiotic therapy postoperatively (see Table 3). However, 3 of the patients in group 3, who had no documented postoperative infection before discharge from the hospital, received 4 or more days of prophylactic antibiotic therapy postoperatively. This intervention may have influenced the results.

Applications of this research would require more in-depth research to isolate the effects of NE dose, decreased blood pressure, and number of ICU days on lymphocyte counts and infection rates in OHSPs. However, the ICU nurse might positively affect the outcome of OHSPs by closely monitoring changes in lymphocyte counts and antibiotic therapy orders of patients exhibiting a profile of increased risk for infection. This study has been beneficial in the identification of one such patient profile. Patients requiring IV NE doses of greater than 0.028 mcg/kg/min for more than 24 h due to hypotension after open heart surgery may indeed be at a higher risk for infection as determined by this study. When applying this study to the assessment of such a patient, the nurse may wish to consider a total circulating lymphocyte count of less than 1100 on postoperative day 2 (see Fig. 2) as an indicator of the increased risk of infection. Additionally, preoperative use of beta-blocking agents may also be considered in the patient with this profile.

Limitations

Since the sample size was limited, N = 75, and the data were collected from only one hospital, caution should be taken before applying these results to all patients. Other limitations to this study include both the variation in preoperative differential counts, manual versus automated, and the inability to measure or control endogenous NE levels in these patients. Further research should be done to confirm this study using several hospitals in different regions for data collection and including a larger patient population.

Additional research should be done limited to the effects of beta-blocking agent therapy on lymphocyte counts of OHSPs receiving NE therapy with a larger population of patients.

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Measurement of the Renin-Angiotensin System in Heart Failure

Shann Dixon Kim, RN, MN

Angiotensin II (ANG II), the effector hormone of the renin-angiotensin system (RAS), has been implicated in the pathophysiology and progression of heart failure. Therefore, the measurement of ANG II has become important to characterize the role of this neurohormone in heart failure. However, because ANG II has been difficult to measure, other components of the RAS have been measured to characterize ANG II production. The RAS components (e.g., renin, angiotensin I–converting enzyme [ACE], angiotensin II) have been measured with a variety of techniques. In this review, RAS physiology and the techniques used to measure the RAS components are discussed. In addition, the advantages and disadvantages of the RAS measurement methods are described.

Key words: *Renin-angiotensin system, heart failure, RAS measurement, renin measurement, ACE measurement, angiotensin II measurement*

The purpose of this article is to review and critique different experimental methods used to measure activation of the renin-angiotensin system (RAS). The effector hormone of the RAS, angiotensin II (ANG II), is involved in the regulation of numerous physiologic processes, such as arterial blood pressure, sodium balance, regional blood flow, and tissue growth (Dzau 1993; Cody 1994). In addition to the role of ANG II in physiologic processes, ANG II has been implicated in the pathophysiology and progression of several diseases, such as heart failure (Lindpainter and others 1992; Dzau 1993; Rousseau and others 1994; Pinto and others 1996; Kawaguchi and Kitabatake 1997; Nicholls and others 1998; Remme 1998). Therefore, because of ANG II's role in physiology and pathophysiology, ANG II measurement has become important.

As discussed in more detail below, ANG II has been difficult to directly measure. Consequently, investigators have measured other components of the RAS, such as renin and angiotensin I–converting enzyme (ACE) to *indirectly* determine ANG II quantities. The other RAS components provide information about the degree of *RAS activation,* or how much ANG II a system can produce. These components have been used to determine the role of ANG II in heart failure and to evaluate the effectiveness of specific pharmacological therapies. The different techniques for measuring RAS activation are discussed, along with methodological considerations and advantages and disadvantages of the different methods. Prior to the discussion of methods, a brief review of the RAS, RAS components, and RAS activation in heart failure is provided.

Components of the RAS and Synthesis of ANG II

Circulating RAS

The circulating RAS pathway begins with the secretion of angiotensinogen from the liver (Fig. 1). The kidneys secrete renin, which hydrolyzes angiotensinogen to angiotensin I (ANG I). As the ANG I circulates throughout the vasculature, in particular, the pulmonary system, angiotensin I–converting enzyme

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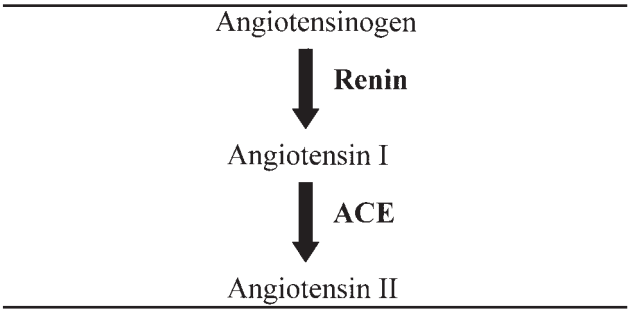

Figure 1. The Circulating Renin-Angiotensin System. The liver synthesizes and secretes angiotensinogen. Renin, which is synthesized and secreted by the kidneys, hydrolyzes angiotensinogen to angiotensin I. Angiotensin I is hydrolyzed to angiotensin II by angiotensin I–converting enzyme, which is found in large quantities in the pulmonary vasculature. Angiotensin II, the effector hormone of the renin-angiotensin system, circulates throughout the body to exert physiologic effects.

(ACE) hydrolyzes the ANG I to ANG II, the effector hormone of the RAS (Sancho and others 1976). Circulating ANG II has short-term physiologic effects on many organs (Table 1) (Csikos and others 1997). Like other endocrine systems, the circulating RAS is subject to feedback control; for example, ANG II inhibits renin secretion from the kidney.

Tissue RAS

In addition to the circulating RAS, a tissue-based RAS has been found in a number of tissues, such as the heart, blood vessels, kidney, and brain (Fabris and others 1990; Weber and others 1992). In the heart, the cellular location of the four main proteins of the RAS (angiotensinogen, renin, ACE, and ANG II receptors) has been investigated (Dzau and others 1981; Lee and others 1996; Neri-Serneri and others 1996). Except for renin, the gene and protein expression of the other RAS components have been found abundantly in the heart. Renin is synthesized in very small quantities in cardiac tissue. Therefore, most tissue renin is believed to be derived from the circulation (Danser and others 1994; Danser and others 1997; Muller and others 1998). A growing number of investigators believe the cardiac tissue RAS, not the circulating RAS, is more important for physiologic *and* pathophysiologic cardiac function (Dzau 1993; Neri-Serneri and others 1996; Cody 1997; Nicholls and others 1998).

Table 1. Physiologic Effects of Circulating Angiotensin II

Organ	Effects
Brain	Increased antidiuretic hormone secretion
Heart	Increased heart rate Increased force of contraction
Blood vessels	Increased vasoconstriction
Kidney	Increased efferent arteriole constriction Increased sodium reabsorption
Adrenal glands	Increased aldosterone secretion

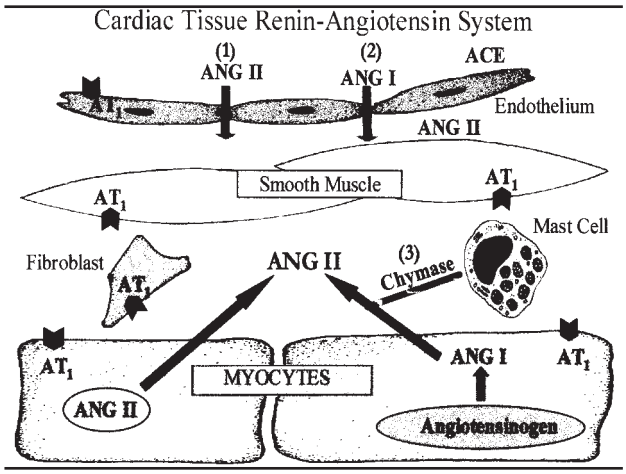

Figure 2. The Cardiac Tissue Renin-Angiotensin System. This figure also shows how ANG II accumulates in cardiac tissue: (1) the uptake of circulating ANG II into cardiac tissue; (2) the conversion of circulating ANG I to ANG II by endothelial cell-bound ACE; (3) the cardiac tissue generation of ANG II by ACE-independent pathways. Abbreviations: ANG I = angiotensin I, ANG II = angiotensin II, ACE = angiotensin I–converting enzyme, AT_1 = angiotensin II type 1 receptor.

As shown in Figure 2, in addition to the tissue synthesis of ANG II by RAS components, ANG II can accumulate within the cardiac tissue in the following ways: (1) circulating ANG II can be directly absorbed from the blood into the tissue; (2) circulating ANG I can be converted to ANG II by cardiac endothelial cell-derived ACE, and subsequently taken up by the cardiac tissue; (3) tissue ANG I can be hydrolyzed to ANG II through ACE-*independent* tissue pathways (Hollenberg and others 1998). To date, the best characterized of the ACE-independent pathways is human heart chymase, which is described below (the ANG II in heart failure section).

Within cardiac tissue, the physiologic effects of ANG II include the local regulation of coronary artery blood flow and stimulation of sympathetic nervous system activity (Dzau 1988; Dzau and Re 1994). Other physiologic effects include stimulation of angiogenesis in utero, but the long-term physiologic effects of tissue ANG II have not been defined, nor have the feedback controls been elucidated.

Angiotensin II Receptors

The physiologic effects of ANG II are mediated through the angiotensin 1 (AT_1) and angiotensin 2 (AT_2) receptors (Timmermans and others 1993). The AT_1 receptor mediates most of the known physiologic effects of ANG II and has been found on endothelial and smooth muscle cells, fibroblasts and cardiac myocytes (Timmermans and others 1993; Suzuki and others 1993; Villareal and others 1993; Sun and Weber 1996; Zisman and others 1998). The widespread distribution of the AT_1 receptor within cardiac tissue suggests significant ANG II–mediated effects through this receptor. Unlike the AT_1 receptor, the distribution and function of the AT_2 receptor are not as well characterized in the heart (Cody 1997). This is probably because the AT_2 receptor is found in much lower quantities than the AT_1 receptor, reportedly about 5-fold less than the AT_1 receptor (Wolf and others 1996).

The ANG II receptors exert distinct physiologic effects and are coupled to different signal transduction systems (Sechi and others 1992; Nio and others 1995; Tsutsumi and others 1998). In fact, many of the physiologic effects of ANG II that are mediated through the AT_1 receptor oppose the effects mediated by the AT_2 receptor (Nahmias and Strosberg 1995). For example, when ANG II binds to the AT_1 receptor, cardiac fibroblast proliferation is *stimulated*, whereas if ANG II binds to the AT_2 receptor, fibroblast proliferation is *inhibited* (Gibbons and Dzau 1994; Stoll and others 1995).

The different physiologic effects are the result of the ANG II receptors operating through different signal transduction pathways. Specifically, the AT_1 receptor has been shown to signal through the phosphoinositide system, whereas the AT_2 receptor may work through inhibition of the adenylyl cyclase system. However, to date the signal transduction pathway(s) of the AT_2 receptor remain to be defined (Inagami 1999).

The overall physiologic activity of ANG II in the heart is controlled by the expression level (up-regulation and down-regulation) of the ANG II receptors (Matsusaka and Ichikawa 1997).

ANG II in the Pathogenesis of Heart Failure

In heart failure, the cardiac tissue production of ANG II is believed to be a more important contributor to cardiac pathophysiology than circulating ANG II (Dzau and Re 1994; Cody 1997). The increased levels of ANG II in cardiac tissue exert long-term, detrimental autocrine and paracrine changes on myocytes and neighboring cells, such as fibroblasts. These cell changes include myocyte hypertrophy, fibroblast proliferation, myocyte necrosis, and myocyte apoptosis (or programmed cell death) (Anversa and others 1991; Meggs and others 1993; Kim and others 1995; Dimmeler and others 1997; Tan and others 1991; Annarosa and others 1998).

Apoptosis (also called cell suicide) is an active type of cell death that is dependent on a genetically encoded pathway. Under physiologic conditions, such as during embryogenesis, apoptosis is essential for the normal cardiac development (Bursch and others 1990). However, under pathophysiologic conditions, such as heart failure, genes involved in apoptosis have been shown to be up-regulated, and apoptosis is believed to contribute to the structural and functional changes associated with heart failure (Olivetti and others 1997). A variety of stimuli are known to stimulate apoptosis. For example, in the heart, increased plasma levels of the cytokine, tumor necrosis factor-alpha (TNF-α), have been shown to induce apoptosis in myocytes (Irwin and others 1999).

Myocyte hypertrophy and fibroblast proliferation increase the mass of the ventricle and lead to ventricular hypertrophy. When myocytes are injured and/or die (e.g., postinfarction), the inflammatory events that follow stimulate fibroblast proliferation. As the fibroblasts proliferate, more collagen is produced, interstitial fibrosis increases, and ventricular hypertrophy ensues (Sun 1997). Over time and depending on the wall stress to a region, the hypertrophied ventricle may begin to dilate, with the intraventricular chambers becoming larger relative to the size of the ventricular wall (Gerdes and Capasso 1995; Amos and White 1997).

Myocyte apoptosis results in the loss of the total number of cells in the ventricle and is associated with *myocyte slippage*. Myocyte slippage occurs when the myocyte position, which is normally fixed, becomes unstable. The myocyte moves as the result of decreased structural support from collagen and the loss of surrounding myocytes, which maintain their position. Along with these changes, the mass of the ventricle decreases relative to the left ventricular cavity, and the ventricle becomes dilated (Anversa and others 1991; Francis and Chu 1995; Gerdes and Capasso 1995). Collectively, these changes in cardiac structure are usually associated with a decrease in contractility and, consequently, low cardiac output and tissue perfusion (Pfeffer and Braunwald 1990; Beltrami and others 1995; Francis 1998).

The pathophysiologic effects of ANG II are inhibited by several peptide systems, in particular, the natriuretic peptides (NPs): atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP). Both NPs have been shown to inhibit the RAS by decreasing the secretion of renin from the kidney and inhibiting the effects of ANG II through the AT_1 receptor (e.g., tissue growth). The NPs inhibit the RAS through signal transduction pathways, which have not been delineated. In addition, the general physiologic effects of the NPs oppose those of the RAS and include increased water and sodium excretion and systemic vasodilation (de Bold and others 1996).

Measurement of RAS Components

In this section, each of the RAS components and the methods commonly used to measure them will be described. Several of the methods have been used to measure more than one RAS component (Tables 3 and 4). To determine RAS activation, investigators have measured either the activity of the enzymes (e.g., renin or ACE) in the RAS pathway or the protein levels of all RAS components (e.g., angiotensinogen, renin, ANG I, ACE, ANG II).

Angiotensinogen

Unlike the other RAS components, angiotensinogen has been measured in very few heart failure studies. The relationship between angiotensinogen and heart failure is unclear because a number of physiologic variables stimulate angiotensinogen secretion, such as increased levels of estrogen. In one study, Arnal and others (1991) reported that angiotensinogen levels were decreased in New York Heart Association (NYHA) class IV heart failure patients. They suggested that the decreased quantities of angiotensinogen reflected high levels of active renin and reduced hepatic output of angiotensinogen. They related their findings to the impact of angiotensinogen levels on renin measurement, not as an independent measure of RAS activation. Because angiotensinogen has been investigated in a very limited number of cardiac-related studies, no further discussion will follow.

Renin: Physiology and Measurement Issues

Renin has been used more than any of the other RAS components to characterize circulating ANG II quantities (Sealey and others 1972; Sealey and Laragh 1996). An aspartyl protease, renin is secreted from the juxtaglomerular (JG) cells and hydrolyzes angiotensinogen to ANG I (Sealey and others 1977). Renin is secreted in 2 forms: prorenin and renin. The active form, renin, is generally considered the rate-limiting step of circulating ANG II formation (Sealey and Laragh 1975). The ratio of prorenin to renin is highly variable, and reports suggest that human plasma has 5-10 times more prorenin than renin (Sealey and others 1977; Campbell and others 1991). Once secreted, the circulating prorenin does not appear to be activated under physiologic blood conditions (pH 7.35-7.45, and 37 °C) and therefore is probably not important for circulating ANG II synthesis (Sealey and Laragh 1996).

Orthostasis, β-adrenergic stimulation and blockade, and diuretics are variables known to influence the prorenin-to-renin ratio (Buhler and others 1972; Sealey and Laragh 1996). In pathological conditions, such as diabetes mellitus, the prorenin quantities can exceed renin 100-fold (Franken and others 1990). The physiologic effects of prorenin are not understood, although some investigators have proposed prorenin may have low intrinsic activity and vasodilating properties (Heinrickson and others 1989; Sealey and Rubatto 1989; Trenkwalder and others 1996).

Although the in vivo conversion of prorenin to renin is unlikely, in vitro (test tube) prorenin conversion to renin has been demonstrated (Sealey and Laragh 1977;

Sealey 1991). Cold temperatures and decreased pH are known to convert the prorenin to renin and increase the amount of measured active renin. If the plasma sample is obtained from a patient with a large quantity of prorenin (e.g., a diabetic patient), even a small amount of conversion could substantially increase the amount of active renin. Prorenin cryoactivation occurs when prorenin is converted to renin by cold temperatures, between 6°C and –20°C (Sealey and Laragh 1996).

Renin Measurement

Under most physiologic (and pathophysiologic) circumstances, renin is the rate-limiting step of circulating ANG II formation (Sealey and Laragh 1977). Therefore, renin activity values are believed to reflect the capacity of the circulatory system to generate ANG II. The most common methods of renin measurement have been the *plasma renin activity* (PRA) and, more recently, the *renin immunoradiometric assay* (IRMA). The PRA measures the rate of ANG I formation from angiotensinogen by active renin, and values are expressed as the amount (nanograms [ng], 10^{-9} grams) of ANG I produced per milliliter of blood per hour (ng/ml/h). The ANG I is quantified with radioimmunoassay (RIA) (Sealey and Laragh 1977). RIA is a very sensitive method used to quantify peptides and proteins, especially when found in small quantities (Thomson and Berry 1996). *Sensitivity* refers to the ability of a method to detect small quantities of a molecule, which for the purposes of this article are the RAS components. The RIA is based on the competition of a radiolabeled peptide and unlabeled peptide binding to a limited number of binding sites on antibodies specific for the peptide of interest. As the concentration of the unknown (unlabeled peptide) increases, the amount of labeled peptide able to bind the antibody decreases. By measuring the amount of radiolabeled peptide, the amount of unlabeled peptide (the quantity of peptide in a specimen) can be determined from a standard curve with known quantities of peptide.

The renin IRMA uses antibodies to directly quantify active (renin) and inactive forms of renin (prorenin), and it is expressed as picograms (pg), which is 10^{-12} grams/ml blood (Morganti, Pelizzola, and Mantero 1995). The IRMA assay involves the reaction of an excess of labeled antibody with the ligand (e.g., renin) of interest. The 2-step or sandwich IRMA is most commonly used and requires 2 specific antibodies raised against epitopes (the part of an antigen that binds an antibody) of the target molecule. The 1st of the antibodies is attached to a solid phase and is used to capture the ligand (e.g., renin), whereas the 2nd antibody is radiolabeled and acts as the "detector" molecule. After a washing step, the amount of ligand can be quantitated. As such, the IRMA is more sensitive than RIA. Furthermore, although the IRMA and RIA are highly sensitive techniques, they recognize immunological activity and may detect denatured, thus *inactive*, protein (Thomson and Berry 1996). Although the PRA and the renin IRMA techniques use different units and procedures, both are closely correlated and represent the same parameter, that is, the number of active renin molecules.

Physiologic variables that affect renin secretion. Since the early 1970s, the PRA is the most widely used measure of RAS activation in heart failure and other cardiovascular-related investigations (Morganti and others 1995a; Sealey and Laragh 1996). However, numerous physiologic variables affect the amount of active renin secreted. These variables must be considered when evaluating or interpreting renin measures (Table 2).

Blood collection and processing of renin. To ensure that renin is accurately measured, several important steps are required (Sealey and Laragh 1977). For baseline renin measurement, the patient should be untreated (i.e., no medication) for 3 weeks prior to blood collection, and on a normal sodium diet. A day or two before the venipuncture, a 24-h urine should be collected to determine sodium excretion and to provide an estimate of sodium balance. The patient should be ambulatory for at least 30 min before the blood draw (the patient may be seated for the venipuncture).

Whether the blood sample will be used for the PRA or renin immunoreactivity assay, the in vitro conversion of prorenin to renin must be prevented. The inadvertent cryoactivation of prorenin could significantly alter the prorenin-to-renin ratio, erroneously decrease the prorenin-to-renin ratio, and increase PRA values. Prorenin cryoactivation can be successfully avoided if the specimens are collected in room temperature vials, centrifuged at room temperature, frozen in liquid

Table 2. Physiologic Variables That Affect Renin Secretion

Variable	Effect	Mechanism of Action
Posture		
Supine	↓active renin	Minimal SNS stimulation. Decreased release of NE from SNS terminals near the JG cells of the kidney.
Upright	↑active renin	Increased SNS stimulation. Increased NE in JC cell region.
Diet		
Low Na^+	↑active renin	Decreased NaCl delivery to macula densa (Loop of Henle), renin secretion stimulated.
High Na^+	↓active renin	Increased NaCl delivery to macula densa, renin secretion inhibited.
Medication		
Diuretics	↑active renin	Decreased total blood volume, SNS stimulated.
ACE inhibitors	↑active renin	Decreased ANG II quantities, lose negative feedback to JG cells.
β-blockers	↓active renin	SNS inhibited.

NOTE: SNS = sympathetic nervous system; NaCl = sodium chloride; β-blockers = β-adrenergic blockers; ACE = angiotensin I–converting enzyme; ANG II = angiotensin II; JG = juxtaglomerular, SNS = sympathetic nervous system.

nitrogen, and stored at temperatures between –40°C and –80 °C. The blood specimen should *never* be placed on ice. If the blood is processed at room temperature and rapidly frozen, the prorenin cryoactivation temperatures will be avoided (Sealey and Laragh 1977). (If liquid nitrogen is unavailable, the specimens can be frozen in a –40 °C freezer.)

Advantages and disadvantages of renin measurement. The measurement of renin has several advantages and disadvantages (Table 3). If the patient preparation and blood collection procedures are followed, the renin measures are sensitive and reliable (Sealey and Laragh 1996; Alderman and others 1997). However, the reproducibility of renin measures between laboratories has been low because the well-characterized guidelines for patient preparation and blood collection have not been followed. In addition, the physiologic variables, which affect renin secretion, such as the patient's medication regimen and diet, are difficult to control. In particular, few heart failure patients are able to stop medication (e.g., diuretics, β-blockers) before blood collection.

Table 3. Methods Commonly Used to Measure Plasma RAS Components

Component	Assay	Advantages and Disadvantages
Renin	PRA—renin activity is measured and quantified with an ANG I RIA	ADV: Most commonly used RAS measure. Incubation easy, rapid. DIS: Depending on the RIA used, can be time-consuming. Requires radionuclides. Numerous physiologic variables affect measures (Table 1).
	IRMA—the amount of renin protein is measured	ADV: Improved renin antibodies, highly sensitive measurement. DIS: Requires radionuclides, time-consuming and expensive.
ACE	Spectrophotometry Spectrofluorometry Both methods used to determine rate of enzyme activity	ADV: Both assays are rapid, reliable. Spectrofluorometric assay more sensitive. DIS: Sensitivity determined by reagents used in assay. Low reproducibility between laboratories because different reagents used.
	HPLC followed by RIA of ANG I and ANG II to determine ANG I : ANG II ratio	ADV: Most accurate measure of ACE activity. DIS: For RIA, radionuclides required. For RIA, ANG II values at the lowest limits of detection. ANG II antibodies cross-react with other angiotensins and artificially increase ANG II values. HPLC is a cumbersome assay. Very time-consuming, expensive, specialized equipment and training required.
ANG II	RIA HPLC followed by RIA	ADV: Most accurate reflection of RAS activation. DIS: Same disadvantages as above for RIA and HPLC.

NOTE: ACE = angiotensin I–converting enzyme, ANG I = angiotensin I, ANG II = angiotensin II; HPLC = high-performance liquid chromatography; IRMA = immunoradiometric assay; PRA = plasma renin activity; RIA = radioimmunoassay.

Although the PRA is the most widely used measure of RAS activation, the renin IRMA may become more popular as the antibodies for renin are further refined (Plouin and others 1990; Simon and others 1992; Morganti, Pelizzola, and Mantero 1995; Morganti, Pelizzola, Mantero, and Gazzano 1995). One of the major obstacles to implementing the IRMA into clinical practice has been the specificity of the antibodies for active renin. Historically, the renin antibodies had large cross-reactivity with prorenin and therefore did not provide an accurate measure of renin. *Cross-reactivity* refers to how specific an antibody is for a particular antigen. For example, if an antibody has high cross-reactivity, the specificity of the antibody for an antigen is low and the antibody will also bind to structurally related molecules. Ideally, an antibody will not react with anything but the molecule of interest and will have low cross-reactivity with other molecules. Recently, investigators have reported increased sensitivity and low cross-reactivity of the renin IRMA (Zuo and others 1992; Morganti, Pelizzola, and Mantero 1995). However, considerable controversy continues to surround the sensitivity of both renin measures (Sealey and others 1994; Menard and Guyene 1995; Morganti, Pelizzola, and Mantero 1995; Morganti, Pelizzola, Mantero, and Gazzano 1995; Sealey and Laragh 1995).

Angiotensin I–Converting Enzyme: Physiology and Measurement

In addition to renin, ACE has been used to indirectly determine ANG II quantities. A zinc metalloenzyme, ACE hydrolyzes the last 2 C-terminal amino acids, histidine (His) and leucine (Leu), of ANG I to form ANG II (Fig. 3). The main substrate for ACE is ANG I; however, ACE acts on a number of other substrates (Skidgel and Erdos 1987). For example, ACE also degrades bradykinin, a potent vasodilator (Beneto and others 1986). Some of the benefits and side effects of ACE-inhibitor therapy are attributed to the increased quantities of bradykinin in cardiac tissue (Carretero and others 1981). In the heart, bradykinin dilates coronary arteries and may inhibit fibroblast proliferation, and thereby decrease left-ventricular hypertrophy (Cody 1994, 1995). However, the overall contribution of bradykinin to the beneficial (e.g., decreased left ventricular hypertrophy) and detrimental (e.g., cough) effects of ACE-inhibitors has not been clearly delineated.

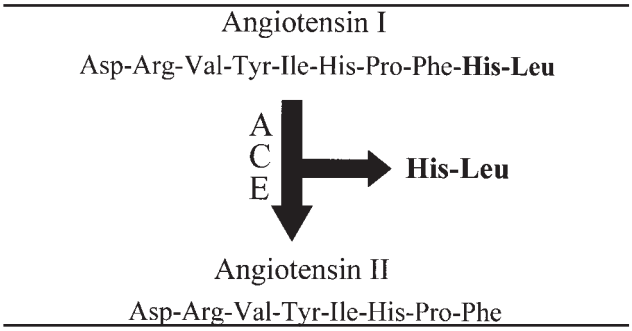

Figure 3. The Conversion of ANG I to ANG II by (ACE). This figure shows the hydrolysis of ANG I to ANG II by ACE. ACE hydrolyzes the N-terminal amino acids, histidine (HIS) and leucine (LEU), to form ANG II. ANG I = angiotensins I, ANG II = angiotensin II, ACE = angiotensin I–converting enzyme.

Some ACE circulates in the blood (called plasma ACE), but the majority of ACE is bound to cell membranes (herein referred to as tissue ACE). Tissue ACE has been localized to the apical membrane of endothelial, epithelial, and neuroepithelial cells, with the majority found on endothelial cells (Ryan and others 1975). The highest concentrations of tissue ACE are found in the kidney, with smaller quantities in the ileum, duodenum, uterus, and lungs (Cushman and Cheung 1971a).

The mechanisms involved in plasma ACE formation have yet to be determined. Plasma ACE may be a product of tissue ACE that was cleaved from the membrane, or plasma ACE may be independently synthesized and secreted by a cell, and therefore not cleaved from existing tissue ACE (Meng and Oparil 1996).

ACE-Independent Pathways of ANG II Formation

In the past 10 years, tissue ANG II has been shown to be produced through ACE-dependent and ACE-independent pathways in the human heart (Balcells and others 1997). Increased interest in the role of ACE-independent pathways resulted from observations that plasma levels of ANG II were elevated in heart failure patients despite long-term ACE-inhibitor therapy (Rousseau and others 1994). Moreover, although ACE inhibitors have proven beneficial in

heart failure patients, the morbidity and mortality are still high in treated patients (Swedberg, Eneroth, Kjekshus, and Wilhelmsen 1990; St. John Sutton and others 1997).

Some investigators believe the ACE-independent pathways, not the ACE-dependent pathway, may be responsible for the majority of cardiac tissue ANG II production in pathological conditions (Okinishi and others 1993; Urata and others 1995; Nishimura and others 1996). Specifically, *human heart chymase* has been shown to be the most specific and catalytically efficient ANG II–forming enzyme yet described (Urata and others 1990; Kinoshita and others 1993; Wolny and others 1997). In addition to the specificity of heart chymase for ANG I, the enzyme is located primarily in the ventricles, which contrasts with ACE whose quantities are highest in the atria. Furthermore, heart chymase is synthesized and secreted by cardiac mast and endothelial cells, a location that is ideal for ANG II production within cardiac tissue (Fig. 2).

However, the role of human heart chymase in ANG II formation remains controversial (Studer and others 1994; Kokkonen and others 1998). Studer and others (1994) demonstrated that patients with end-stage heart failure have increased ACE activity compared with healthy patients. These investigators used the polymerase chain reaction (PCR) to amplify and determine the gene expression of major ANG II–forming enzymes (ACE and chymase) in the left ventricle. The amount of ACE transcription was increased 3-fold in patients with chronic heart failure compared with nonfailing hearts. No difference was found in the gene expression of chymase between the failing and nonfailing hearts. Although the Studer and others (1994) study suggested that ACE may be more important in tissue ANG II production, the roles of ACE-dependent and ACE-independent pathways have not been resolved.

Methods of ACE Activity Measurement

Because ACE generates ANG II in the RAS pathway, changes in ACE activity or the amount of ACE protein theoretically reflect quantitative changes in the amount of ANG II generated in the circulation or cardiac tissue. Accordingly, ACE activity and ACE protein levels have been used as markers of ANG II formation in plasma and tissue (Bruckschlegel and others 1995). In heart failure, a number of human and animal studies have shown increased ACE activity (Pinto and others 1991; Paul and others 1992). ACE gene expression (messenger RNA, mRNA) has been measured in very few studies because the quantities of cardiac ACE mRNA are extremely small, even in pathological conditions. Reverse-transcriptase polymerase chain reaction (RT-PCR) has been required to generate sufficient quantities for analysis; therefore, the clinical and experimental usefulness of ACE mRNA measurements are limited (Lear and others 1997).

Of the ACE measures, ACE-activity assays have been used most often to determine circulating and tissue RAS activation (Tables 3 and 4). ACE activity assays measure the amount of ANG II formed from ANG I (Fig. 3). Few studies have measured the amount of ACE protein in the heart or circulation.

A number of methods have been reported to determine ACE activity. These methods have been used to characterize RAS activation and to evaluate ACE-inhibitor therapy in humans and animals. In ACE-activity assays, an aliquot of serum or tissue (the source of ACE) is added to a test tube, which contains a natural or artificial substrate that can be cleaved by the endogenous ACE. The use of artificial substrates (the molecules that an enzyme acts on) has largely replaced the natural substrates, ANG I and bradykinin. The artificial substrates, usually tripeptides, are hydrolyzed to dipeptides that can be easily detected with spectrophotometric and spectrofluorometric methods (Fig. 4). The spectrophotometric method of Cushman and Cheung (1971b) is the most common method of ACE activity measurement. In this method, an ACE-specific substrate, hippuryl-histidyl-leucine (HHL), is degraded by ACE to form a reaction product, hippuric acid (HA). The amount of HA produced by ACE is detected and quantified with a spectrophotometer.

A modification of the Cushman and Cheung spectrophotometric method uses spectrofluorometry to measure ACE activity and has been used in a number of studies to determine ACE activity (Friedland and Silverstein 1976). In this method, a fluorescent tag (usually o-phthaldialdehyde) is added to a tissue or plasma specimen. Then, the fluorescent tag forms a complex with the His-Leu that has been cleaved by the endogenous ACE. With the spectrofluorometer, the amount of fluorescent His-Leu can be quantified.

Table 4. Methods Commonly Used to Measure Tissue RAS Components

Component	Assay	Advantages and Disadvantages
ACE	Spectrophotometry Spectrofluorometry Both methods used to determine rate of enzyme activity	ADV: Both methods frequently used. Rapid, relatively inexpensive. Small quantities of tissue can be used in spectrofluorometric assays. DIS: Assay reagents affect values. Low reproducibility between laboratories because different reagents are used.
ANG II	RIA HPCL with RIA	ADV: Most accurate reflection of RAS activation. RIA is a sensitive, reliable measure for low peptide quantities. HPLC powerful method for separation of angiotensins. DIS: ANG II antibodies cross-react with other angiotensins. Requires radionuclides. HPLC is cumbersome, expensive, time-consuming and requires trained personnel.

NOTE: ACE = angiotensin I–converting enzyme; ANG II = angiotensin II; HPLC = high-performance liquid chromatography; RIA = radioimmunoassay.

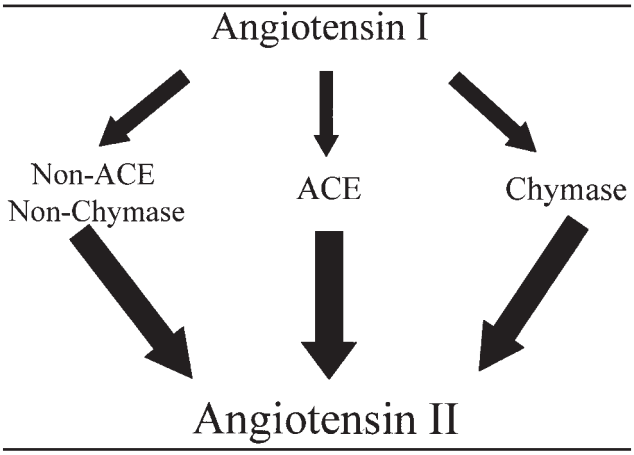

Figure 4. Possible Enzymatic Pathways of Angiotensin II Formation in the Heart. ACE = angiotensin I–converting enzyme.

These methods have been used extensively in animal and human models of heart failure, but the measurement of ACE activity has several limitations (Tables 3 and 4). If an investigator wants to determine ACE activity, both the plasma and tissue ACE should be measured. This is because the mechanisms that regulate both ACEs are not known and may be different (Meng and Oparil 1996). Thus, both plasma and cardiac tissue measures of ACE activity will more accurately reflect the circulating and tissue changes in heart failure.

An alternative approach to the enzymatic measurement of ACE is to directly measure the plasma and tissue concentrations of ANG I and ANG II to obtain a ratio of the ANG I to ANG II. The ANG I-to-ANG II ratio is considered the most accurate reflection of ACE activity. Some investigators have even recommended the use of the ANG I-to-ANG II ratio to monitor patient compliance with ACE-inhibitor treatment (MacFayden and Struthers 1997).

The ANG I and ANG II quantities have been measured with RIA. RIA has been used alone, or in combination with high-performance liquid chromatography (HPLC) to quantitate ANG II in heart failure. HPLC is a highly sensitive method used to separate amino acids, peptides, and proteins, and it is the most widely used method to purify and separate peptides. To purify a peptide from other structurally similar peptides, a standard (e.g., ANG II) is injected onto an HPLC column and the elution time determined by a computer. Based on the time of elution (i.e., when the peptide of interest comes off of the HPLC column into a collecting tube), samples from a specimen can be collected, either manually or automatically. HPLC is an extremely powerful method to separate structurally similar compounds, which may be indistinguishable using other methods (Thomson and Berry 1996). Whether plasma or tissue specimens are used, HPLC can fractionate the angiotensins into ANG I, ANG II, ANG III, and other small angiotensin metabolites. Then, RIA is used to quantitate the angiotensin fractions of interest.

A major limitation of the ANG I-to-ANG II ratio is that the RIA used to measure the ANG II is close to the lower limits of detectability of RIA (Tables 3 and 4). Consequently, other peptides may interfere with ANG II measurement. In particular, angiotensin III (ANG III), a metabolite of ANG II, with plasma quantities of

about one-half of ANG II (Campbell and others 1991), has 100% cross-reactivity with the ANG II antibody. Thus, ANG II RIA measures alone will be higher than the actual ANG II values. Several individual laboratories have reportedly synthesized ANG II antibodies with high specificity for ANG II and little cross-reactivity to ANG III. However, to date, the commercially available antibodies still have 100% cross-reactivity with ANG III. Another limitation is that HPLC requires very expensive instrumentation and is labor-intensive. Many laboratories do not have access to HPLC, or personnel trained to operate the instrument. Consequently, the widespread measurement of the ANG I : ANG II ratio is unlikely.

Other ACE Activity Considerations

In addition to the measurement issues associated with ACE activity methods, theoretical issues must be considered when ACE activity measures are used to determine circulating and/or tissue ANG II. First, plasma ACE activity has high intersubject variation with wide ranges in ACE activity demonstrated within the same patient populations (Fogarty and others 1989). Second, increased plasma ACE activity is associated with a number of clinical conditions and may not be a sensitive measure of cardiac function (Delacretaz and others 1994). Third, although cardiac tissue samples can be easily obtained from animals at the time of sacrifice, in humans, cardiac tissue measurement of ACE activity has been more difficult. In nearly all human studies that have measured cardiac tissue ACE activity, tissue was obtained from explanted or postmortem hearts. The explanted hearts are available only from individuals with end-stage myocardial disease; therefore, true pathological tissue changes may be obscured. In addition, the control donor hearts are typically procured from brain-dead individuals who were exposed to a number of factors (e.g., multiple drug therapy) that could interfere with reliable biochemical measurement after explantation (White and others 1995). Recently, investigators have shown that biopsy-sized cardiac tissue specimens could be safely obtained from nonfailing, hypertrophied, and failing hearts (Lowes and others 1997).

In addition to the preceding ACE-activity considerations, several other considerations must be mentioned. Tissue ACE is not evenly distributed across tissues, especially a heterogeneous tissue like the heart. Thus, a sample obtained from one part of an infarcted left ventricle may not accurately represent the entire ventricle. Also, fibrotic tissue has been shown to have more ACE than nonfibrous tissue (Weber and others 1995; Ou and others 1996). If the sample is collected from a section of ventricle without fibrosis, absent or low ACE activity would not reflect the increased ACE activity in other parts of the injured ventricle. Therefore, consistent sampling from the same part of a cardiac chamber is important for comparisons of tissue ACE activity between experimental groups. Finally, cardiac tissue ACE activity may not reflect the primary source of ANG II formation, particularly in humans, because ACE-independent pathways may be more important.

Angiotensin II: Physiology and Measurement

Because ANG II can be formed through pathways other than the ACE-dependent pathway, the direct measurement of ANG II is the most accurate to determine the physiologic and pathophysiologic role(s) of ANG II in the heart. ANG II is an 8 amino acid peptide, normally found in low pg quantities (10^{-12} moles), around 2 to 10 pg/ml of blood in humans and 4 to 20 pg/ml blood in rats (Nussberger and others 1989). In addition, ANG II has a half-life of about 12 s in the blood and 2 to 3 min in vitro (Braget and others 1997). Closely related inactive precursors and metabolite peptides (e.g., ANG I, angiotensin (4-8) pentapeptide) are also present in the plasma in considerable quantities (Moeller and others 1998). To date, most studies have measured ANG II in plasma; few studies have examined cardiac tissue quantities of ANG II (Sun and others 1997; Leenen and others 1999).

Historically, the direct measurement of ANG II has been difficult because the peptide is found in very small quantities, even when the RAS is activated (Berecek and Zhang 1995). In addition, ANG II has a short half-life and is easily degraded by angiotensinases in the blood. Two methods have been used to directly quantify ANG II: RIA and HPLC (Tables 3 and 4). As mentioned in the ACE measurement section, the most commonly reported method for ANG II measurement has been ANG II RIA alone, without

HPLC separation of the angiotensins. Because the plasma and tissue quantities of ANG II are below the limits of HPLC to quantify ANG II, HPLC may be used to reliably separate the angiotensins, which can then be quantified with RIA. ANG II values derived by HPLC separation of the angiotensins followed by RIA are considered highly accurate (Braget and others 1997).

As mentioned earlier, direct ANG II measurement has not been widely used because the methods have high cross-reactivity with ANG II metabolites (RIA) and are cumbersome (HPLC) (Tables 3 and 4). Moreover, if inhibitors of ANG I (e.g., ACE inhibitors) and ANG II (e.g., angiotensinase inhibitors) have not been added to a specimen, the formation of ANG I and ANG II will continue after collection and increase ANG II values. To further complicate the measurement, if blood samples are obtained from humans or animals receiving ACE inhibitors, the levels of ANG II are so low (femtograms, 10^{-15} g) that they become undetectable with current methods. Finally, cardiac tissue measures of ANG II may not reflect actual tissue synthesis because tissue ANG II values probably reflect both the amount of ANG II taken up from the circulation and tissue synthesis.

Clinical Applications of RAS Measurement

The important role of ANG II in heart failure has been corroborated by numerous animal and human studies (Swedberg, Eneroth, Kjekshus, and Snapinn 1990; Swedberg, Eneroth, Kjekshus, and Wilhelmsen 1990; Rouleau and others 1991; Tan and others 1991; Kabour and others 1994; Huttl and others 1995; Kim and others 1995; Weber and others 1995; Lear and others 1997; Remme 1998; Carraway and others 1999). In humans, several large-scale clinical trials of heart failure demonstrated improved function, regressed hypertrophy, and increased survival in patients treated with ACE-inhibitors, which block the formation of ANG II (Swedberg and Kjekshus 1987; Swedberg, Eneroth, Kjekshus, and Snapinn 1990; Swedberg, Eneroth, Kjekshus, and Wilhelmsen 1990; Studies of Left Ventricular Dysfunction Investigators 1991, 1992; Pfeffer and others 1992; Ball and others 1993; Rouleau and others 1994).

Heart failure patients with increased plasma levels of neurohormones, such as ANG II, have been shown to have a poor prognosis (Swedberg, Eneroth, Kjekshus, and Wilhelmsen 1990; Rouleau and others 1991). In recent years, the RAS components have been measured in several of the large-scale heart failure trials as potential biochemical markers of heart failure. In the Cooperative North Scandinavian Enalapril Survival Study (CONSENSUS) trial, elevated baseline ANG II values were correlated to increased 6-month mortality after enalapril treatment (Swedberg, Eneroth, Kjekshus, and Wilhelmsen 1990). Another longitudinal study, the Survival and Ventricular Enlargement (SAVE) trial reported that increased baseline PRA was correlated to 1-year mortality and total CV mortality (Rouleau and others 1994). On the other hand, the Studies of Left Ventricular Dysfunction (SOLVD) trial (1992) reported no change in the PRA of asymptomatic or symptomatic heart failure patients who did not receive diuretics, and they concluded that PRA was not associated with overall mortality or mortality from progressive heart failure. Recently, in another subset of cardiovascular patients, Alderman and others (1997) found that before the initiation of pharmacological treatment for hypertension, the PRA levels of hypertensive patients were independently and directly associated with the incidence of myocardial infarction.

The discrepant results from these and other studies have several possible explanations. First, the heart failure patient populations in the clinical trials were heterogeneous, with differences in the severity of their heart failure (e.g., NYHA class) and pharmacological regimens (e.g., diuretics, β-blockers). For example, all subjects in the CONSENSUS I trial received diuretic therapy, which increases plasma renin (both prorenin and renin activity levels). Also, if the subjects adhered to a low-sodium diet, PRA values would have been further increased. Because the medication and diet regimes of the patients had potentially activated the RAS before participation in the trials, none of the RAS components would provide prognostic information.

In addition to the subject-related variations, the methods that were used to measure the RAS components may have contributed to the variability. In the CONSENSUS I trial, ANG II was measured with direct RIA; HPLC separation of the angiotensins was

not performed before the RIA. Moreover, the RIA used by the investigators had 100% cross-reactivity with ANG III, which was likely to have resulted in erroneously high ANG II values. Furthermore, both the SAVE and SOLVD trial investigators reportedly placed the blood specimens for PRA on ice after venipuncture. Consequently, prorenin cryoactivation could have artificially increased the PRA, or at the very least, increased variability of the PRA results. The amount of prorenin cryoactivation could have been substantial depending on the length of time the specimens were kept at cryoactivation temperatures, and the underlying pathophysiology of the subject (e.g., diabetes mellitus). In addition, patients in the SOLVD trial had been in a supine position at least 30 min before venipuncture. Collectively, the degree of RAS activation in the patients from the clinical trials is difficult to ascertain because the patient preparation, blood-processing conditions, and methods in these studies were not optimal or consistent.

Although the observations from the large heart failure clinical trials are not proof of ANG II's role in cardiac pathophysiology, the competitive receptor antagonist, losartan, an AT_1 receptor blocker, has provided further support. In the Evaluation of Losartan in the Elderly (ELITE), losartan was shown to have similar or greater efficacy than the ACE-inhibitor, captopril, in reducing overall mortality in persons over the age of 65. In addition to the improved mortality over captopril, losartan was generally better tolerated, and fewer patients discontinued losartan therapy (Pitt and others 1997). These results suggest that if ANG II is produced through ACE-dependent and ACE-independent pathways, then direct blockade of the AT_1 receptor would be more advantageous than ACE inhibition alone.

Conclusions and Recommendations

Few would dispute a role for ANG II in the pathophysiology and progression of heart failure. However, the results from the heart failure clinical trials and other studies have shown that the methods currently used to measure the RAS components may not be sensitive or reliable enough for widespread use as biochemical markers of heart failure severity and progression. In addition, several of the methods, which do provide sensitive measurement (e.g., RIA and HPLC) are not accessible to most clinicians.

The RAS is activated by a number of physiologic and pathophysiologic conditions (Table 2). Therefore, studies that measure RAS components to predict disease progression or outcomes should be carefully evaluated to ensure that optimal specimen preparation and processing conditions were used to prevent inaccurate results. Furthermore, RAS measures should be used with other more reliable biochemical markers, such as the NPs, ANP and BNP, to corroborate research findings. The NPs have been correlated with heart failure severity and shown useful to stratify patients and monitor pharmacological therapy (Madsen and others 1995; Lainchbury and others 1997; Cheung and Kumana 1998; Maeda and others 1998; Sagnella 1998).

The RAS components may have a prognostic future in heart failure if new, more sensitive methods are developed to detect changes in these proteins. In particular, techniques are needed to accurately quantify tissue ANG II from small amounts (e.g., biopsy) of cardiac tissue. All of the RAS components (angiotensinogen, renin, ACE, AT_1 and AT_2 receptors) and heart chymase have been cloned. Therefore, as molecular biology techniques improve, mutated RAS components may be identified as risk factors for heart failure. Gene polymorphisms (variations in a gene sequence) that occur of the RAS components, ACE, human heart chymase, and the AT_1 receptor have been examined in heart failure patients. Some of those studies have demonstrated an association of the polymorphisms with cardiac pathology, but the results have not been consistent (Cambien and others 1992; Cambien and Evans 1995; Raynolds and Perryman 1995; Pfeufer and others 1996; Candy and others 1999; Vancura and others 1999).

On the other hand, as ACE-independent pathways of ANG II generation are better characterized, the classical RAS components may not prove as important for ANG II production in the heart. As mentioned previously, heart chymase has been shown to be a potentially important ACE-independent pathway of ANG II formation (Urata and others 1995; Wolny and others 1997). Other non-ACE, nonchymase enzyme pathways may also be involved (Fig. 4). The relative

importance of these enzyme pathways to the overall generation of ANG II in the human heart must be delineated. Given the importance of ANG II in the pathophysiology and progression of heart failure, sensitive and clinically useful methods to detect and monitor ACE-dependent and/or ACE-independent pathways of ANG II generation are imperative.

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Basic Science Research in Pain

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Pain is not simply a physiologic event, but a dynamic process that involves continuous interaction among complex systems. Nurses have the unique disciplinary background to envision the pain process within the context of the whole dynamic human being. Understanding the human condition in this holistic manner prepares nurses to develop clinically relevant research questions. Many of these research questions can best be answered initially through basic science research. Basic science research by a nurse will be distinct from other disciplines from the inception of the hypothesis through the conclusions drawn to the delineation of areas for further research. This article provides a few examples of basic science research in the area of pain by nurse researchers. The research includes both cellular and animal models and describes the relationship of the research to clinical practice. Patient care will ultimately benefit from clinically relevant research whether the methodology used is basic science or other methods.

The human pain experience is a complex interwoven combination of physiological processes, subjective experiences, culture, psychological attitude, and multiple other variables. In essence, pain perception is affected by and affects the whole person: body, mind, and spirit. One argument for nurses being involved in basic science research on pain is their holistic perspective. They are able to envision these physiological processes within the context of the whole dynamic human being. Because pain has widespread clinical significance, and treating pain is a nursing responsibility, it is essential that nurses research and understand pain mechanisms and interventions. Although the physiologic aspects of pain are only one of several influences affecting the human pain experience, basic science research, by nurses, in this area can provide knowledge to facilitate more satisfactory pain management.

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The ability to effectively treat pain still alludes health care practitioners. For example, from 1973 to 1998, researchers reported that more than 40% of patients suffer from moderate to severe acute postoperative pain (Cronin and others 1973; Cohen 1980; Warfield and Kahn 1995; D'Arcy and Ebner 1998). Concomitantly, chronic pain afflicts millions of people, and relief for most is inadequate. Estimates suggest that approximately 30% of the population in industrialized countries have chronic pain, disabling them from weeks to permanently, making chronic pain not only an individual health problem but a socioeconomic one costing society $13 billion in health care expenses alone (Bonica 1990).

Treating pain is not just a humane or ethical matter. Pain has biological consequences that are disruptive to homeostasis. The pathophysiological changes that occur with acute pain include increased sympathetic tone and hormonal and metabolic derangements. Poorly managed perioperative pain control can lead to a cascade of negative patient outcomes including increased length of stay in hospital, patient dissatisfaction, and delay in resuming activities of daily living. Chronic pain is associated with decreased quality of life, depression, fatigue, decreased mobility, and sleep disturbances (Hitchcock and others 1994), as well as prolonged reaction time and interference with task performance (Eccleston and others 1997). More important, pain can lead to immune suppression and tumor metastasis (Page and others 1993).

Occasional postoperative patient complaints of severe pain, which was only moderately relieved by potent opioids, led me to investigate this phenomenon. A review of the literature revealed that these patients may have been suffering from hyperalgesia, a state in which the intensity and duration of response to painful stimuli are enhanced (Woolf and Thompson 1991;

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Doughtery and Willis 1992; Mayer and others 1998). Glutamate receptors play a major role in hyperalgesia, thus my research interest in these receptors.

In this article, a background for understanding glutamate receptors and their relationship to pain transmission is provided, followed by a rationale for basic science research by nursing. Examples of research of involving glutamate receptors, pain mechanisms, and outcomes in both cellular and animal models and their relationship to clinical practice are then described.

Background

The most ubiquitous excitatory neurotransmitter within the central nervous system is glutamate. Glutamate has activity in the spinal cord and higher brain regions and plays a crucial role in both acute and chronic pain transmission. There are 2 general types of glutamate receptors, ionotropic and metabotropic (Hollmann and Heinemann 1994). Ionotropic glutamate receptors are ligand-gated ion channels. The ionotropic glutamate receptors can be pharmacologically categorized into N-methyl-D aspartate (NMDA) and non-NMDA glutamate receptors. The 2 non-NMDA receptors, α-amino-3 hydroxy-5 methyl-4 isoxazole proprionic acid (AMPA) and kainate (KA), regulate a sodium/potassium channel, which mediates excitatory postsynaptic potentials (Corderre and Melzak 1991; Furuyama and others 1993), whereas NMDA receptors are calcium channels that play a major role in hyperalgesia. Whereas non-NMDA ionotropic glutamate receptors play a role in "normal" pain transmission, NMDA receptors are the catalysts for hyperalgesia, by allowing the passage of extracellular calcium into the cell. This large influx of calcium activates calcium-dependent second-messenger cascades resulting in nitric oxide (NO) production, protein kinase C (PKC) activation, and prostaglandin metabolism. Figure 1 depicts a schematic of the NMDA receptor.

Until recently it was thought that glutamate activated ligand-gated cationic channels only. However, Sladeczek (Sladeczek and others 1995) reported that glutamate stimulated phospholipase C by a receptor that was not ligand-gated. The 1st metabotropic glutamate receptor, 1a (mGluR1a) was cloned in 1991 (Houamed and others 1991; Masu and others 1991).

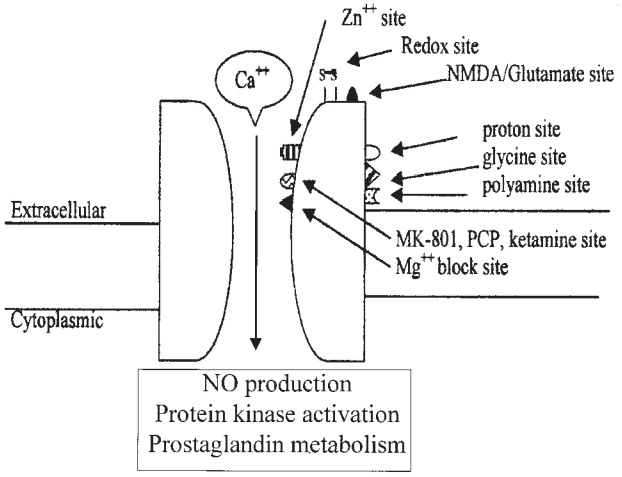

Figure 1. A schematic representation of an NMDA receptor, indicating sites for various agonists, antagonists, and modulators.

The structure and function of mGluR1a has not been fully explicated, but current research provides evidence that the receptor is involved in the transmission of acute and chronic pain (Neugebauer and others 1994; Young and others 1995).

The metabotropic glutamate receptors belong to the superfamily of G-linked proteins. These receptors are not channels, but transduce a message intracellulary when activated. When a ligand binds to the MGluR1a receptor, it causes a conformational change in the receptor that affects the G protein associated with it. The G protein dissociates from the receptor and causes a cascade of events that leads to an alteration in intracellular signaling molecules. Most notable is the increase in intracellular calcium. Figure 2 demonstrates a schematic of metabotropic glutamate receptors 1 and 5.

It is becoming increasingly clear that metabotropic glutamate receptors, most particularly mGluR1a and 5, act in conjunction with ionotropic glutamate receptors to mediate pain transmission. Understanding how pain is transmitted is the first step in being able to control it.

Rationale for Basic Science Research by Nursing

Basic science research by a nurse is research that is distinct from other disciplines in that the inception of the hypothesis, the conclusions drawn, and the

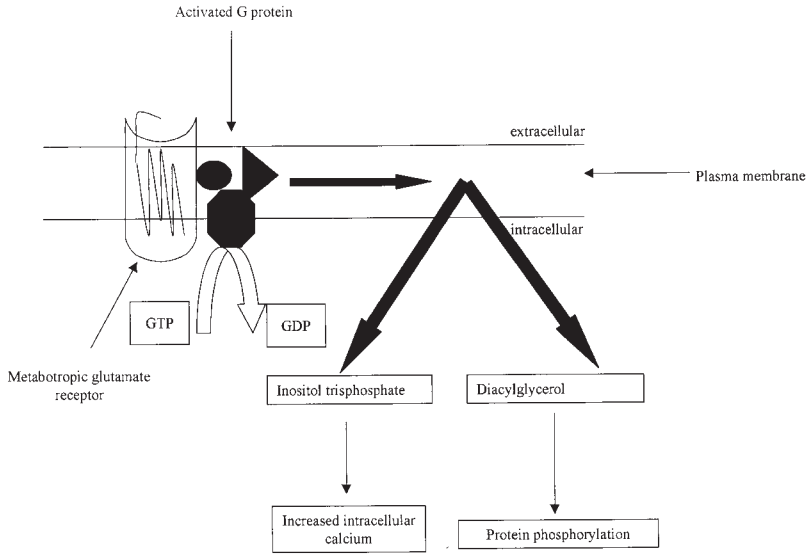

Figure 2. A schematic representation of metabotropic glutamate receptors 1 and 5 indicating second messenger pathways and products.

delineation of areas for further research are from a nursing perspective. The nurse researcher looks at research in terms of health, environment, person, and nursing and makes inferences from this perspective. Other scientific disciplines do not have the same questions that nursing has and so are not likely to investigate the same topics that nurse researchers choose (Donaldson and Crowley 1978). If nursing waits for other disciplines to pursue particular topics of inquiry that are best studied in the laboratory, those topics may never be researched because the question may not be germane to other disciplines. The question asked is the most crucial aspect of the research and determines whether the knowledge gained from that research would fall within nursing's domain, not the methodology.

It is possible to maintain the vision of the holistic person and still value basic science research. It is possible to ask a nursing question that needs some further physiological evaluation before taking that research forward for clinical study. This is the case of the research by Gayle Page, DNSc. It had been known since the early 1980s that surgery suppressed natural killer (NK) cell activity (Pollock and others 1984). Natural killer cells are a subpopulation of large granular lymphocytes that are thought to be the first line of defense against virally infected cells, several other types of infections, and some types of tumor cells. Dr. Page, a nurse researcher, first suggested that the pain of undergoing and recovering from surgery may be a factor in surgery-induced suppression of natural killer cell activity (Page and others 1993; Page and Ben-Eliyahu 1997). With her, I have been studying the effects of pain on natural killer cell activity via the activation of glutamate receptors in whole animals.

Prior to the research with Dr. Page, I began cellular research on the metabotropic glutamate receptor 1a in the laboratory of Gerda Breitwieser, PhD. Because metabotropic glutamate receptors are a new area of research, available pharmacological compounds are not specific for each subtype of receptor, nor are they approved for human use, thus limiting research to cellular, tissue, or whole animal research. Because the compounds are not specific, when they bind to the receptor, all or most of the 8 subtypes of metabotropic glutamate receptors are activated. This creates confounding variables in in vivo studies, leading to uninterpretable results. To overcome this, the mGluR1a receptor was transfected into a cell line so that only that receptor will be activated upon drug induction.

Cellular Approach to the Study of Glutamate Receptors and Pain Transmission

Cell culture is particularly useful in describing ligand receptor interactions and kinetics. It has 3

distinct advantages. The physiochemical environment can be closely controlled, the samples are homogenous, and modulation by other competing receptors can be controlled. Two constructs of mGluR1a have been engineered for stable transfection into HEK-293 cells, a line of human embryonic kidney cells. The pBlue Script plasmid containing mGluR1a was obtained (Thomas P. Segerson, Oregon Health Sciences University) and digested with NOT I to excise the receptor DNA. This fragment was gel purified and ligated into P-EGFP, an expression vector for mammalian cells that has a green fluorescent protein (GFP) incorporated into its DNA. P-EGFP was also digested with NOT I. The same vector, P-EGFP, was used for both constructs to control for variations in response to drug that may be vector related. By fusing the C terminus of the mGluR1a to the N terminus of the GFP, a chimeric protein was produced that, when stably transfected and expressed by the cell line, will fluoresce. Tarasova (1997) used this technique to study G protein-coupled cholycystokinin receptor A localization and trafficking. Cholycystokinin receptor A is similar to mGluR1a in that signal transduction is via inositol hydrolysis. The GFP did not alter ligand binding affinity, signal transduction, or expression. The 2nd construct is similar to the 1st except that the GFP has been excised using SACII restriction enzyme endonuclease. Both constructs have been confirmed by restriction enzyme digestion which produced the predicted bands. Further, segments of the constructs were sequenced by polymerase chain reaction (PCR) which produced the predicted DNA sequences and both have been transiently transfected into HEK-293 cells with transfection confirmed by immunofluorescence (see Figure 3). Stable cell lines are being screened. Once a stable cell line is established, characteristics of the receptor can be examined.

Although using a cellular paradigm to study the effects of the mGluR1a receptor limit's generalizability, at present there are no specific agonists or antagonists to simplify in vivo studies. Therefore, in vitro studies can more clearly elucidate the structure and function of this receptor. Understanding the structure and function of the receptor is critical for rational drug design and development. A comprehensive characterization of the receptor will permit the development of highly selective drugs that would affect a limited area of the central nervous system. Pharmacological tools

Figure 3. The recombinant rat metabotropic glutamate receptor 1a/GFP expressed in HEK-293 cell. Light portions of the photograph indicate successful transfection of the receptor/green fluorescent protein molecule.

of this type would presumably be more precise and have fewer side effects than the current glutamate antagonists. As newer and more specific agonists and antagonists are developed, these agents can be used to elucidate more precisely the role of metabotropic glutamate receptors in pain transmission. Subsequently, selective antagonists can be generated for clinical trials.

Whole Animal In Vivo Approach to the Study of Glutamate Receptors and Pain Transmission

NK cells are one of the body's first defenses against metastatic spread of cancer (Lotzová 1991), yet the activity of these cells is suppressed by a major treatment for cancer, surgery (Pollock and others 1984; Pollock and Lotzová 1987; Pollock and others 1989, 1991, 1992; Page and others 1994; Page and Ben-Eliyahu 1997). The mechanism by which surgery suppresses NK cell activity has not been fully elucidated, but one hypothesis is that the pain of undergoing and recovering from surgery can suppress the activity of NK cells and thereby increase the risk of tumor metastasis (Page and others 1993; Page and others 1998). Using an experimental metastasis model in adult male rats, it was shown that surgery enhances the metastasis of an NK-sensitive mammary adenocarcinoma (Page and others 1994). Furthermore, the metastatic-enhancing effects of surgery were shown to be ameliorated by the administration of an opioid analgesic (Page and

Table 1. Experimental Design for Each of the 4 Pilot Assays

Treatment	Males			Females		
	Vehicle	Low dose	High dose	Vehicle	Low dose	High dose
Surgery + Anesthesia						
Anesthesia only						

others 1993). However, morphine administration did not completely reverse this life-threatening consequence of surgery, suggesting other pain mechanisms are involved.

With the support of Dr. Page, I am exploring the effects of modulation of the ionotropic glutamate NMDA receptor in ameliorating the metastatic-enhancing effects of surgery using an experimental metastasis model in adult male and female Fischer 344 rats. A stratified, randomized $2 \times 2 \times 3$ factorial design has been employed such that both Fischer 344 male and female rats undergo either a standard laparotomy with halothane anesthesia or anesthesia only and receive either NMDA modulator or vehicle.

Pilot assays were conducted to explore the effects of 2 modulators of NMDA activity on surgery-induced decreases in host resistance against metastasis. To accomplish these pilots, a $2 \times 2 \times 3$ design was used: males versus females by surgery with anesthesia versus anesthesia alone, by the vehicle versus low versus high drug doses. Each assay used a total of approximately 40 rats, n = 2-5 per cell (see Table 1).

The pilot studies suggested that NMDA modulators alone are not effective in ameliorating surgery-induced resistance against metastasis and may even be detrimental.

The accompanying release of dopamine that occurs in certain areas of the brain with the administration of NMDA modulators might explain these findings. Increased brain dopamine levels have been associated with NK cell activity suppression. The results of the pilot studies support the necessity of animal research prior to human clinical trials by suggesting that FDA-approved drugs in this paradigm used as preoperative medication in humans may suppress NK cell activity. Because of these results, the research has been redesigned using NMDA modulators to supplement opioids. It is expected that, in combination with an opioid, a smaller dose of NMDA modulator can be used, thus circumventing the effects of dopamine release and providing increased resistance against surgery-induced experimental metastasis.

Using an animal model allows for better experimental control than is attainable in human studies. In particular, the surgery is the same for all animals, a surgery shown to result in NK suppression and the promotion of metastasis (Page and others 1993; Page and others 1994). The administration of the glutamate receptor modulators can be regulated experimentally and compared against unmedicated control groups and morphine only groups. The outcome variables are objective, quantifiable, and tangible evidence of tumor load and nociceptive efficacy.

Findings from the research may be significant for several reasons. Millions of people undergo surgery each year and any intervention shown to be efficacious in reducing a risk such as immune suppression would impact a very large number of people. If NK cell activity plays a role in inhibiting spontaneous metastasis in humans, and surgery suppresses NK cell activity, then any intervention shown to ameliorate surgery-induced suppression of NK cell activity may potentially decrease the risk of spontaneous metastasis and increase long-term survival. The costs of cancer metastasis are enormous, not only in terms of human lives, but in diverting dollars from other uses to health care. A study examining costs of colon, prostate, and breast cancer showed that the direct costs of these cancers, if localized, were approximately $31,000 per individual for 6 months of care, whereas once the cancers spread, costs rose significantly to $44,000 per individual (Taplin and others 1995). Results of this research have the potential to support and extend findings implicating pain in the negative immune and metastatic consequences of undergoing and recovering from surgery. Such findings can empower health care providers by providing a physiological foundation on which to advocate for effective postoperative pain management and on which to base postoperative pain management strategies for optimizing surgical outcomes. A key aspect of the anesthesia nursing care of an individual recovering from surgery is pain management. If the findings of this study are ultimately supported by clinical trials, this research has the

potential to change anesthesia practice and postoperative care of patients.

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How Enteral Feeding Options Influence Corticosterone Patterns in Rats

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Even with all the nutritional research conducted to date, it is not clear which enteral nutrition delivery and composition options are most physiologically sound. Glucocorticoid temporal patterns are reported to be shifted or disrupted with restricted feeding schedules, but because of intermittent sampling, temporal patterns have not been completely depicted. The purpose of this study was to characterize corticosterone temporal patterns while systematically varying selected enteral feeding options in a well-established nutritional animal model. A 2 × 2 × 2 × 2 randomized block experimental design was used in which enteral feeding schedules, delivery methods, kilocalorie levels (kcal), and fiber contents were systematically varied in rats ($n = 80$*), and plasma corticosterone was measured by* ^{125}I *radioimmunoassay. Blood samples were drawn hourly over 24 h. With cosinor analysis, 24-h and 12-h corticosterone rhythmic components were tested in each feeding group. Five of 16 feeding groups had a significant (*$p \leq 0.05$*) 24-h rhythmic component, and 3 more showed a trend (*$p > 0.05 < 0.10$*); 7 of these groups were on 24-h feeding schedules. When rhythmic components were detectable, groups receiving high-fiber formula displayed more uniform rhythm characteristics than did no-fiber groups. Only 1 group had a significant 12-h rhythmic component, and 1 showed a trend. Both were on 12-h, high-fiber restricted kcal feedings. In this small animal sample, no one enteral feeding option guaranteed a 24-h corticosterone pattern. The option coming closest was formula delivered on a 24-h schedule. This temporal pattern is one aspect to consider in enteral nutrition. The underlying mechanisms have yet to be elucidated.*

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In patients for whom food access, digestion, or absorption is disrupted by disease, treatment, or age, the risk that nutrients will not keep pace with metabolic demands is high (Marvin 1988; Bergstrom and Braden 1990; Klein and others 1997). When the gastrointestinal tract remains functional, the vast majority of these patients can be treated with oral or enteral nutrition to maintain weight and improve nutritional status (Stotts 1990).

Enteral nutrition, in which nutrients are delivered directly to the stomach or small intestine, bypassing the mouth, is being prescribed at an unprecedented rate in both the hospital and home (Rombeau and Rolandelli 1997). From 1989 to 1992, the number of Medicare patients on home enteral feedings doubled, and the average home patient received 70 days of such feedings. In 1992, about 152,000 home patients were receiving enteral feedings (Howard and others 1995).

There are many enteral feeding options; they vary in different formula ingredients (Lord and others 1996) including fiber levels (Homann and others 1994), as well as in delivery methods and schedules (Beau and

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Labat 1994; Forloines-Lynn 1996). Feeding volumes and subsequent caloric intake can be adversely affected by slow infusion rates (Lord and others 1996) and mechanical problems with feeding tubes (Heyland and others 1995).

These variations in delivery schedules and methods, kilocalorie (kcal) levels, and fiber contents have resulted in many physiological changes, some with untoward health and nutritional consequences (Rombeau and Rolandelli 1997). However, diverse samples and differences in the variables manipulated and measured, as well as the analysis approaches used, make it difficult to compare many study results of liquid nutrition. Confusion arises in part from the fact that several studies have examined selected aspects of enteral feedings without accounting for other aspects. Because it is not consistently explicit which nutritional option or combination of options resulted in the reported changes, there is not yet a clear basis for making sound decisions on enteral nutrition delivery. Thus, the study described here systematically investigated outcomes of selected aspects of enteral feedings to contribute to the physiological base for optimal enteral nutrition. This report presents findings on one such outcome, glucocorticoid temporal patterns.

Glucocorticoids

Cortisol is the primary human glucocorticoid; corticosterone, the rat form. Glucocorticoids are essential for cellular metabolism, as well as for mounting and sustaining a response to stress. They have been credited with influencing multiple body functions and processes (Loriaux and Cutler 1986; Hadley 1996), including aspects of immunity, as well as selected mRNA synthesis for such products as substance P, a neuropeptide widely distributed in the body that is thought to be active in processes such as pain (Malendowicz and others 1996).

In addition to its aperiodic release in response to stress, there is a basal glucocorticoid component. The pulsitile release (Reynolds and others 1980; Windle and others 1998) gives rise to the body's well-established circadian, or 24-h, rhythm that peaks at or near activity onset. The presence of this rhythm has been linked positively with health (Arendt and others 1989; Office of Technical Assessment 1991). However, conditions such as shift work and some diseases and treatments result in rhythmic disruption of glucocorticoids (Arendt and others 1989; Moore-Ede 1993; Lanuza 1995).

Time Structure

A time structure exists in biological systems and is detectable by regularly recurring variations manifested as rhythms (Walker and others 1990). Common rhythmic properties include period length, amplitude, peak time, and mean level. The time structure can link several rhythms together, producing sets of synchronized rhythms. Such synchronization is thought to be a prerequisite for health and feelings of well-being (Moore-Ede and others 1982). Well-documented rhythms, such as body temperature or glucocorticoids, may act as marker rhythms for less well studied or detectable rhythms within the larger time structure (Walker and others 1990). Thus, accurate characterization of a corticosterone temporal pattern under varied enteral feeding conditions may contribute to a more accurate depiction of other processes displaying temporal fluctuations that are directly, or coincidentally, related to glucocorticoid levels.

Nutrition and Corticosterone Rhythms

If an individual's physiology changes during the course of a day, logically the same environmental condition may lead to varied body responses throughout the day. Human food intake is one such condition. It normally occurs intermittently during waking hours. Episodic food access has been found to alter corticosterone temporal patterns in rodents (Kreiger 1974; Nelson and others 1975; Wilkinson and others 1979; Armario and others 1987; Sander 1992; Westfall and Heitkemper 1992). When food access was restricted, corticosterone levels often adjusted with a peak level around the time of food availability. Unfortunately, most samples were obtained intermittently, often at 4- to 12-h intervals. Thus, although variations could be detected, a temporal pattern could not be accurately characterized. Additionally, because of limited feeding time, the amount consumed was calorically deficient in some studies. Thus, caloric inadequacy or food constituents may have played a part in corticosterone temporal pattern shifts or disruptions. Therefore, this study was designed to characterize daily temporal

patterns of corticosterone in rats while systematically varying enteral feeding schedules, delivery methods, kcal levels, and fiber content. Although rats are nocturnal, they are a well-accepted nutritional model.

Method

Design

To minimize the impact of aberrations that could occur during the 93 days of data collection, a $2 \times 2 \times 2 \times 2$ randomized block experimental design was adopted for this animal model enteral feeding study. The study was approved by the Oregon Health Sciences University Animal Care Committee.

Sample

Eighty healthy Sprague-Dawley postpubescent male rats (Simenson Laboratory, Gilroy, CA) completed the study. Seven additional animals were lost to the study from vascular surgery complications on study days 16-20. Entry weights on the day of gastrostomy surgery ranged from 170 to 191 g ($X \pm SD$ 181 ± 5.6 g). Each rat was in the study for 21 days following this surgery. Analysis of variance showed no significant daily weight differences ($p \geq 0.05$) among feeding groups during the first 6 postsurgery days. Holding constant the kcal intake for postsurgery days 6 to 9, there were no significant differences ($p \geq 0.05$) in body weight by group on postsurgery day 10, the last day before enteral feedings were started. Thus, the body weights in all feeding groups were similar at the start of enteral feedings on day 11. Five animals completed each of the 16 treatment cells.

Independent Variables

Each of the 4 nutritional variables had 2 levels: (a) feeding schedule period (24-h and 12-h [rest time]); (b) delivery method (infusion pump and syringe bolus); (c) daily kcal level (80 and 55); and (d) fiber content (Jevity = 3.4 g/240 ml [high] and OsomliteHN = 0.0 g/240 ml [none]). The variables and levels were chosen to reflect current clinical practices in enteral nutrition. For the 12-h schedule, feedings were delivered during usual rest time, that is, when the lights were on. Enteral feedings are supplied to patients using a continuous or intermittent approach. Intermittent feeding can be further delineated by the number and time interval over which food is delivered. During a 24-h period, 80 kcal have been shown to be enough to maintain a steady weight gain (Westfall and Heitkemper 1992). The restricted level of 55 kcal/24-h was expected to better approximate the equivalent of what patients often receive when feedings are disrupted (Koruda and others 1987). The fiber levels were those from 2 commercial liquid formulas used with patients. The formulas, Jevity and OsmoliteHN, were isotonic, lactose-free protein isolates with the same caloric distribution of 16.7% protein, 30.0% fat, and 53.3% carbohydrate (Ross Laboratories 1992). The study sequence and feeding groups are summarized in Figure 1.

Experimental Procedures

At all times, rats were treated in a manner consistent with the "Guide for the Use and Care of Laboratory Animals," approved by the Council of the American Physiological Society. To allow for animal adjustment to study protocols and synchronization to the environment, acclimatization was planned. All animals were housed in a room reserved exclusively for this study in an approved care facility. The 12:12 (12 h on, 12 h off) lighting cycle was controlled by an automatic timer. Lights came on at 0600 h and went off at 1800 h. Dim red illumination was used in the room when working after the lights were off. Room temperature and humidity were controlled, continuously measured, and recorded daily.

Throughout their stay, rats were weighed daily during the first 2 h the lights were on. To minimize the release of corticosterone in response to stress, animals were handled gently at least twice a day by project staff, using standard protocols (Westfall 1993). Initially, animals were housed in groups of 3 to 4 per wire-bottom cage with ad libitum water and solid food. After 5 to 7 days, rats were transferred to single, wire-bottom cages with ad libitum water. Food consistency was changed from solid to liquid. If randomized to the no-fiber groups, 80 kcal of OsmoliteHN was provided daily; if to the high-fiber groups, 80 kcal of Jevity. Half the food was available when the lights came on; the other half, just before the lights went off.

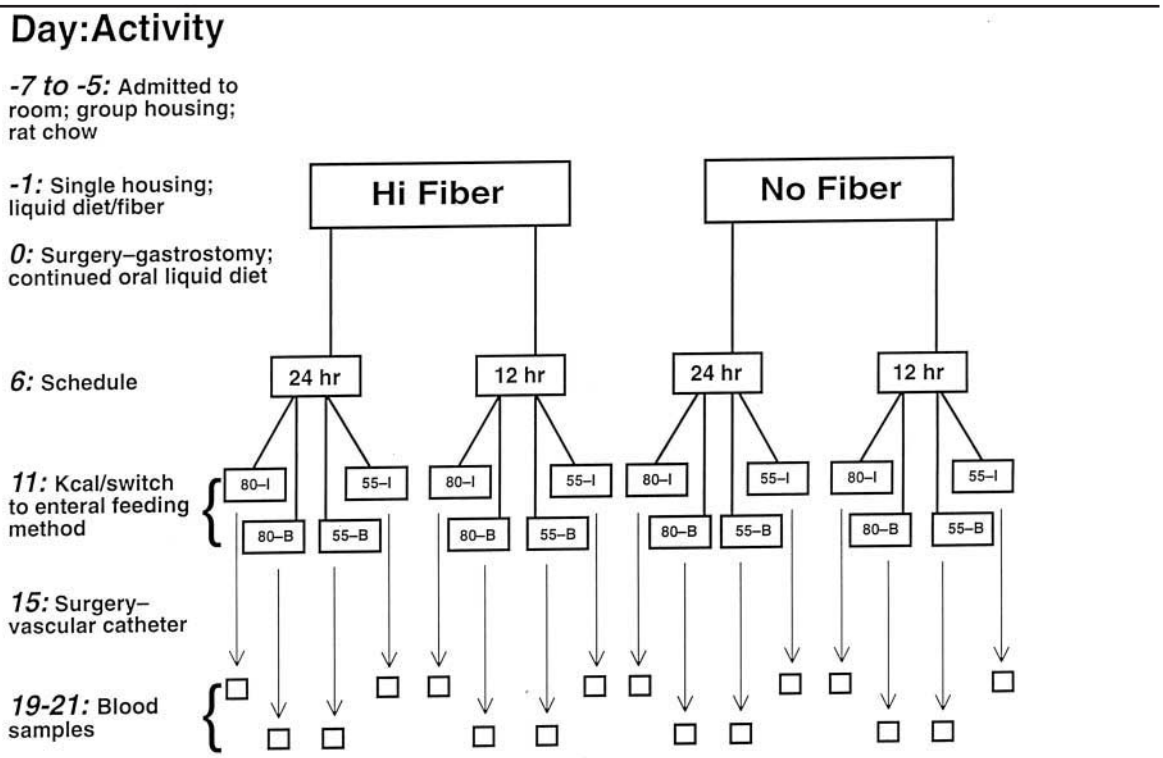

Figure 1. Temporal study sequence listing day in room with major activities to the left and group breakdown by independent variable levels to the right.

NOTE: There were 16 feeding groups on postsurgery day 11. Through postsurgery day 10, all rats had access to 80 kcal/day. 80 = 80 kcal and 55 = 55 kcal/day; I = infusion pump; and B = bolus delivery.

Within 1 to 2 days after cage transfer, all gastrostomy surgery was performed by the PI under sterile conditions with inhalation anesthesia (halothane), for placement of a permanent tube (PE190). The tube was tunneled subcutaneously from the stomach to a suprascapular exit site and then sealed with a removable pin. Following recovery from anesthesia, liquid formula was available, and continued on the 24-h oral schedule. On postsurgery day 6, rats randomized to the 12-h feeding schedule began receiving food only from 0600 to 1800 h, the time lights were on in the room. By postsurgery day 9, the kcal intake was not significantly different ($p \geq 0.05$) from that of rats with food available around the clock.

Enteral feedings began on postsurgery day 11 and continued through postsurgery day 21. Food was delivered either by infusion pump or by syringe bolus. To maintain consistent time intervals for bolus-fed animals, food was delivered to each rat in the same sequence over 1 to 3 min. For rats on a 12-h bolus schedule, feedings were every 4 h, beginning at 0600 h. For those on a 24-h schedule, bolus feedings were every 3 h around the clock, also beginning at 0600 h. Additionally on day 11, rats randomized to the lower kcal level were reduced from 80 to 55 kcal/24-h. Rats could freely move within their cages whether receiving food by infusion or bolus method.

On postsurgery day 15, a carotid artery catheter (PE 50) was surgically placed for vascular access on postsurgery days 19-21. A dilute heparinized saline solution (200 units/1 ml) filled the catheter, which was sealed with a removable pin. Catheters were checked and flushed daily, with dead space refilled with heparin solution.

After 4 full days for recovery, hourly blood samples were drawn, filling 2-3 heparinized capillary tubes per sample. In each feeding group, 24 samples were obtained, 1 for each hour. Occasionally, 2 samples were available for a single time point. When possible, the 2nd sample was timed to be drawn around the time

lighting changed. When present, the 2 values were comparable. Because the lighting phases changed at 0600 and 1800 h, samples were drawn within ±10 min of each hour. Samples were immediately placed on ice.

Within 15 min of being drawn, samples were centrifuged for 4 min using an MHCT II (Model # 0556 Clay Adams, Parsippany, NJ). A hematocrit level was obtained on each sample with a spiracrit wheel (Sherwood Medical Industries, St. Louis, MO). Normal adult rat hematocrit values are 48.1 ± 0.5% (Bruckner-Kardoss and Wostmann 1974). Elevated plasma corticosterone levels can occur in response to low blood volume from multiple sampling. When a rat's hematocrit level reached <39%, no further samples were drawn. No more than 2.0 ml of blood, ~10% of anticipated vascular volume (Bruckner-Kardoss and Wostmann 1974), was obtained from any one rat. Samples from 3 or more rats in each group were needed to complete the 24-h period. Contributing to necessary sample homogeneity were identical age on arrival, sex, and vendor of the rats, as well as a single study room, around-the-clock protocols, and consistent personnel. To accommodate ethical and technical considerations while characterizing the blood corticosterone levels across 24 h, a composite animal for each group was constructed. This strategy is supported by Minors and Waterhouse (1989) and involved having data from different time points derived from different animals within each feeding group.

Plasma samples were stored at –20 °C until processed. All samples were tested in duplicate using commercial double antibody corticosterone ^{125}I radioimmunoassay kits specifically developed for rat and mice plasma (ICN Biomedicals, Inc., Costa Mesa, CA). To minimize variability, all kits had the same stock number and were used within 15 days of shipping. Coefficients of variation were calculated for all assays and were ≤ 3.5%. Radioactive sample counts were converted to µg/dl (100 ml) using ICN IsoDATA software.

Body weights were measured to the nearest 0.1 g using an A&D balance EK1200A (A&D Engineering, Inc., Milpitas, CA). Known weights confirmed instrument accuracy.

Twenty-four hours is the period of the well-established corticosterone rhythm. To determine if a 24-h rhythmic component existed in these rats, cosinor analysis for this period length was computed for each feeding group. Additionally, because all animals were handled when the lights came on and shortly before the lights went off, cosinor analysis was also performed for a 12-h period. Cosinor analysis involves fitting a cosine curve for a rhythm with a known period using least squares regression (Lentz 1990). The analysis is designed to determine whether data have a significant sinusoidal rhythm for a known period, and, if so, to enable calculation of 3 rhythm properties: amplitude (peak-to-trough differences), midpoint (midline estimate statistic of rhythm [mesor]), and acrophase (the curve-fitted peak time). Significance levels were set at $p \leq 0.05$ and at $p > 0.05 < 0.10$ (two-tailed) for a trend toward significance.

Daily kcal intakes and body weights were compared across feeding groups using analysis of variance and analysis of covariance, holding kilocalorie intakes on study days 6 through 9 constant. Multiple comparison corrections were applied when calculating significance levels, $p \leq 0.05$ (one-tailed).

Results

24-h Rhythmic Component

The F ratio and R^2 values for all 16 feeding groups are displayed in Table 1. For the 5 feeding groups with a demonstrated ($p \leq 0.05$), and 3 groups with a suggested ($p > 0.05 < 0.10$), 24-h rhythmic component, the amplitude (peak-to-trough value), midpoint (mesor for cosinor analysis), and acrophase (curve-fitted peak time in hours [military time]) are also presented. All F ratio and R^2 values are included to document that results in the remaining cells rarely come close to significance. Graphed corticosterone levels for one group, with the fitted sinusoidal 24-h curve, are presented in the upper panel of Figure 2.

More peak-to-trough variability occurred among the no-fiber groups than in the high-fiber groups, as displayed in Table 1. Midpoint values of corticosterone 24-h rhythmic components were remarkably low, ranging from 3.3 µg/dl to 6.4 µg/dl. An interesting difference was observed between the infusion and bolus-fed groups receiving 80 kcal, regardless of fiber content. The midpoints were essentially 2 µg/dl higher in the infusion groups (i.e., 5.6 and 5.9 µg/dl) than in the bolus-fed groups (3.7 and 3.8 µg/dl). Curve-fit peak times for the 5 groups with significant rhythmic

Table 1. Detectable Plasma Corticosterone 24-h Rhythmic Components by Feeding Group

Feeding Schedule		Food Delivery			
		80 kcal		55 kcal	
		Infusion	Bolus	Infusion	Bolus
24 hour	High fiber				
	F ratio	2.58	7.61*	5.17*	1.1
	R^2	0.20	0.42	0.33	0.09
	Amplitude (µg/dl)	6.22	5.46	5.87	
	Midpoint (µg/dl)	5.6	3.7	3.6	
	Acrophase (24-h peak time)	1655	2019	1719	
	No fiber				
	F ratio	4.21*	2.69	3.80*	4.05*
	R^2	0.29	0.20	0.28	0.29
	Amplitude (µg/dl)	9.46	4.90	4.85	10.91
	Midpoint (µg/dl)	5.9	3.8	3.3	6.4
	Acrophase (24-h peak time)	2015	2103	1924	1629
12 hour	High fiber				
	F ratio	0.94	0.07	0.09	1.41
	R^2	0.08	0.01	0.01	0.12
	Amplitude (µg/dl)				
	Midpoint (µg/dl)				
	Acrophase (24-h peak time)				
	No fiber				
	F ratio	0.02	2.10	3.27	0.31
	R^2	0.00	0.17	0.24	0.03
	Amplitude (µg/dl)			6.30	
	Midpoint (µg/dl)			4.7	
	Acrophase (24-h peak time)			1759	

NOTE: Lights on between 0600 and 1800 h.
*significant at $p \leq 0.05$.

components fell within a consecutive 3-h and 50-min time span; for all 8 groups, within a 4-h and 34-min time span. The times extended from late-rest through early-activity. Corticosterone peak times before 1800 h occurred in 3 groups on restricted kilocalories but in only 1 group receiving 80 kcal (high-fiber, infusion). For the remaining 80-kcal feeding groups, peak times occurred more than 2 hours after the lights cycled off and within a 48-min span (2015 to 2103 h).

12-h Rhythmic Component

Only 1 group had a significant 12-h rhythmic component ($p \leq 0.05$), and 1 group showed a trend ($p > 0.05 < 0.10$) toward significance. Both received high-fiber, restricted kilocalories on a 12-h schedule. The infusion group demonstrated a significant rhythmic component (*F* ratio = 7.94; $R^2 = 0.43$); the bolus group, a trend (*F* ratio = 3.06; $R^2 = 0.23$). Corticosterone levels for the infusion-fed group are presented in the lower panel of Figure 2.

0600 ± 1 Hour Feeding Time Values

For the 8 groups on 12-h feeding schedules, individual corticosterone values around the time that feedings started are displayed in Table 2. In 6 of the 8 groups, there were values >9.0 µg/dl at 1 or 2 of these hours. Only the high-fiber, 80-kcal infusion group and the no-fiber, 55-kcal bolus group did not have an elevated corticosterone level.

In summary, only half of the 16 feeding groups exhibited a significant 24-h corticosterone rhythmic component (5 groups), or a trend (3 groups) by cosinor analysis. Seven of these groups received feedings on a 24-h schedule. The 5 groups that clearly demonstrated 24-h rhythmic components ($p \leq 0.05$) were on 24-h feeding schedules. There was more variability of 24-h rhythmic properties among the no-fiber groups than the high-fiber groups. Although only 2 groups demonstrated, or showed, a trend toward a 12-h rhythmic component (both of these were on a 12-h feeding schedule), 6 of the 8 groups on 12-h schedules had elevated corticosterone levels around the start of feeding.

Discussion

The aim of this study was to characterize corticosterone temporal patterns with different enteral feeding options in rats. Several possible confounders were taken into account in the study design. The first was stress-induced corticosterone release. The ^{125}I assay measured only total circulating corticosterone concentrations and did not differentiate between the bound and the biologically active unbound forms. With stress events, blood hormone concentrations increase (Hadley 1996). The half-life of cortisol is about 60 min (Loriaux and Cutler 1986); corticosterone has a somewhat shorter half-life (Dallman and Yates 1969). Thus, stressful events within a given hour can influence the blood level for that hour. As noted earlier, standard protocols with handling, as well as a consistent environment and personnel, were maintained throughout

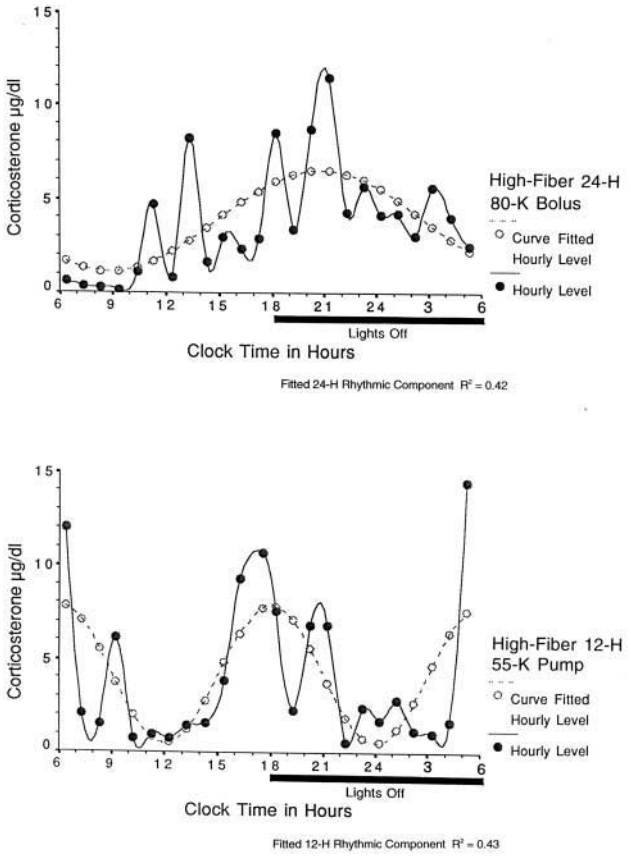

Figure 2. Sequential hourly plasma corticosterone levels in µg/dl (•—•) and cosine analysis curve-fit levels (o--o) for high-fiber, 24-h 80-kcal bolus group (upper panel) and high-fiber, 12-h 55-kcal infusion group (lower panel). Blood drawn at each hour ±10 min. Nocturnal rats begin activity phase when lights cycle off. Both groups had a significant rhythm: upper panel, 24-h; lower panel, 12-h. For 12-h infusion schedule, formula started 0600 h and finished at 1800 h. Note prefeeding elevated values at 0500 and 0600 h.

the study to minimize corticosterone release in response to stress. However, this does not exclude the possibility that aperiodic stress could be reflected in the corticosterone level for any given hour. Finally, a glucocorticoid increase is detectable in blood within 5 min of a stimulus (Reynolds and others 1980). To control for this rapid rise, blood samples were drawn within the first 2 min the rat was handled.

The midpoint corticosterone levels for feeding groups, as shown in Table 1, were consistent with reported mean minimal concentrations in male Sprague-Dawley rats of 1-6 µg/dl, and they were well

Table 2. Single Plasma Corticosterone Values in µg/dl at Lights On ± 1 h for 12-h Schedule Groups

		Food Delivery			
		80 kcal		55 kcal	
Feeding Schedule	Fiber Content	Infusion	Bolus	Infusion	Bolus
12 hour	High fiber				
	1 h before lights on	0.92	0.51	**14.54**	**9.18**
	H lights on	2.31	**26.50**	**12.06**	3.73
	1 h after lights on	2.32	**10.82**	2.06	6.87
	No fiber				
	1 h before lights on	**15.32**	0.99	**16.37**	1.27
	H lights on	0.27	**12.70**	0.37	2.09
	1 h after lights on	1.94	**30.22**	1.28	1.97

NOTE: Lights on between 0600 and 1800 h. Bolded values mean >9 µg/dl.

below mean maximum concentrations, which range from 15 to 23 µg/dl (DePaolo and Masoro 1989). These values lend support that the study maintained a low-stress environment. However, the higher midpoint values in the 80-kcal infusion groups suggest this delivery method is more stressful than bolus delivery of ample kcal levels.

Second, interindividual differences among rats in each feeding group were also of concern, since a composite animal was constructed for each group. Because a single rat's blood volume totals only ~7.08 ml/100 g body weight (Bruckner-Kardoss and Wostmann 1974), no more than 10 samples were drawn from any 1 animal. Thus, samples from 3 or more rats in each group were needed to constitute a 24-h period. To secure a homogeneous sample, all animals were 41 days old when they arrived from a single vendor and were of similar weight at the time of gastric tube placement, the study period was confined to 93 consecutive days, and consistent environmental conditions and standardized protocols were maintained throughout the study. Nonetheless, constructing the composite rat was a compromise. The recent automated sampling system described by Windle and colleagues (1998), enabling large numbers of samples to be obtained from a single animal, could control for possible interindividual differences, but this system was not available when the current study was done. Still, when graphing data points for each feeding group, marked differences among the rats in each group were not detected.

Third, it may be argued that hourly sampling did not produce sufficiently dense data to pick up pulsitile phenomena (Minors and Waterhouse 1989). Monk (1987) reports that infrequent sampling can be used to characterize rhythms as long as the extreme values and slope can be detected. A clear 24-h period for corticosterone has been established. Thus, 24 evenly spaced data points drawn on the hour ±10 min should be sufficient to characterize a 24-h rhythmic component.

Finally, cosinor analysis assumes the presence of a deterministic cycle that is sinusoidal with a known period length, amplitude, and phase (Lentz 1990). Yet, rhythmic phenomena that do not meet these assumptions exist for 24-h periods, as well as other time intervals (Elmore and Burr 1993). Thus, if a rhythm is not detected by cosinor analysis, one cannot conclude that a rhythm does not exist. Additionally, more than 1 rhythm period may exist for a given variable, as exemplified in the work of Windle and colleagues (1998), which showed an hourly corticosterone rhythm in female rats as well as the 24-h pattern. Nonetheless, normal profiles of glucocorticoid rhythms consistently display 24-h patterns resembling sinusoidal waves (Aschoff 1979; Van Cauter 1989; Hastings 1991; Windle and others 1998). Thus, cosinor analysis was appropriate for the data collected here.

These procedural and analysis limitations would apply to all feeding groups equally. Although caution must be exercised when interpreting these findings, in part because of the small sample and composite animal construct, significant rhythmic components were observed in some groups but not others, and there was detection of 24-h and 12-h temporal patterns. These findings would argue for a limited impact of these potential biases.

Several findings from the study are of particular importance for enteral feedings. First, only 5 of the 8 feeding groups receiving enteral feedings on a 24-h schedule showed a significant 24-h temporal pattern. Two additional groups on a 24-h schedule displayed a trend toward significance. It is well established in humans and animals that 24-h glucocorticoid rhythms can be observed with food available throughout the day (Aschoff 1979; Arendt and others 1989). Persistent rhythmic glucocorticoid secretion with varied nutritional intake states and schedules were reported by Van Cauter (1990). However, modulating effects also were mentioned. Such effects may exert stronger or weaker influences on glucocorticoid rhythms depending on the configuration of feeding factors. In the present study, no single enteral feeding option was strong enough to ensure the presence of the established 24-h rhythm, although a 24-h feeding schedule came the closest. Only 1 group receiving food on a 12-h schedule showed even a trend toward a 24-h rhythmic component. This suggests the possibility of temporal pattern-induced differences with enteral feedings, especially those delivered on a 12-h rest-time schedule.

Clearly, the time of day when food is ingested has been shown to influence corticosterone concentrations. Here, elevations in initial perifeeding corticosterone values were observed in 6 of the 8 12-h fed groups, as shown in Table 2. This is consistent with other findings, many using limited time sampling intervals and an oral, rather than an enteral, feeding route. Sander (1992) reported corticosterone levels doubling when fasted rats orally consumed food during the early light cycle. In contrast, corticosterone levels decreased when fasted animals were fed orally during 30 min of the early dark cycle. Kreiger (1974) reported a 12-h shift in basal corticosterone when rats were maintained on rest-time restricted food access. Numerous other studies have shown that corticosterone rhythms may be shifted or split with restricted access schedules (Nelson and others 1975; Honma and others 1984; Oliveira and others 1993; Leal and others 1995). However, others have found that food ingestion led to decreased, increased, or no change in glucocorticoids, depending on the experimental design, age, and species (Brandenberger and others 1982; Sander and Thomas 1988; Sabatino and others 1991; Abrahamsen and Carr 1996; Honma and others 1996). Furthermore, the part that the cephalic digestive phase may play in glucocorticoid dynamics cannot be overlooked, especially since the cephalic phase is bypassed in enteral feedings.

In the current study, the two 12-h groups that did not display a corticosterone elevation at feeding onset ±1 h were opposites on each of the nutritional variables. If elevated corticosterone is a response to the stress of fasting, one would expect a high level in all groups

receiving restricted kilocalories on a 12-h schedule. However, this was not the case. Honma and colleagues (1996) reported that the corticosterone peak did not appear in orally fed rats when meal time was extended to 6 h/day. They concluded that motivation, not the amount of food consumed, was important in prefeeding corticosterone peaks. However, it is unclear how motivation might be measured, if this would differ between animals using the oral versus enteral route, and why it would not be reflected in other feeding groups on 12-h schedules. Although there appears to be no clear explanation for the 2 opposite groups, findings to date support delivery schedule as a factor to consider in glucocorticoid dynamics.

We also found that when rhythmic components were detected or suggested in feeding groups, there were differences in rhythmic properties between the restricted and ample kilocalorie groups. For example, corticosterone peak times differed between these groups. A 24-h glucocorticoid rhythm normally peaks around activity onset, that is, when lights go off. Two of the 3 restricted kcal groups with significant rhythmic components displayed curve-fit peak times before lights went off (that is, 1629 and 1719 h). Such a shift may reflect some food-restriction stress that could become intensified toward the end of the rest period. Two other potential stress sources were the afternoon handling by personnel and the enteral feeding delivery method. However, neither of these sources seems likely as the sole factor because all rats received late afternoon handling, and both infusion and bolus delivery methods were used with both kilocalorie levels. In contrast, in both 80-kcal groups with significant 24-h rhythmic components and a 3rd group with a rhythmic trend, peak times occurred over 2 h after the lights went off. In these groups, with ample kilocalorie intake, there may be delayed arousal in the early activity period. Without further study, it remains unclear whether the later peak times in the ample kilocalorie groups reflect altered internal dynamics of corticosterone production, metabolism, or degradation, or an altered response to exogenous stressor(s).

It has been reported that oral kilocalorie restriction leads to a higher corticosterone level (Leakey and others 1994; Hanson and others 1997). In the present study, the highest midpoint (6.4 μg/dl) for all groups with a suggested or detectable 24-h rhythmic component was found in the restricted-kilocalorie 24-h no-fiber bolus-fed group, supporting the findings of Hanson and colleagues. However, the remaining groups on restricted kilocalorie received formula by continuous infusion and did not manifest a midpoint greater than 4.7 μg/dl. Leakey and her colleagues reported that elevated serum corticosterone levels can exist with restricted calories for relatively short intervals, and may diminish with age. However, the current study sequence lasted only 21 days. This time frame does not exceed the intervals implied by Leakey. Although procedural differences among studies, such as sampling times and frequency, may contribute to conflicting findings, standardized protocols were maintained in the current study. Thus, such technical differences would not explain the varied corticosterone midpoints for the enterally fed restricted kilocalorie groups found in this study.

The delivery method used can contribute to the mass or speed of contents through the gut. There is evidence to suggest that release of selected hormones, such as insulin (Dallman and others 1995), or neuropeptides from the intestine, as well as splanchnic innervation to the adrenal gland, (Dijkstra and others 1996), can influence corticosterone release. Vasoactive intestinal polypeptide (VIP) and met-enkephalin were shown by Hinson and colleagues (1994) to produce a 3- to 4-fold dose-dependent increase in corticosterone secretion; substance P and neurotensin produced a smaller response—less than a 2-fold increase.

The underlying cellular mechanisms responsible for the different corticosterone levels and rhythmic characteristics were not examined in this study. However, the fiber content of formula, as well as the delivery method, may be important in this regard. Inhibition of proximal and distal small intestinal transit has been reported in response to carbohydrate and fat, respectively (Lin and others 1996; Lin, Zhao, and Wang 1997). Additionally, fiber has been shown to slow the movement of enteral formula through the small intestine by activation of the distal "ileal brake" (Lin, Zhao, Chu, and others 1997). It is thus possible that pace or speed of contents through the small intestine, or gut distention, plays a role in the activation or release of corticosterone. Further exploration of possible links between such enteral neuropeptides, small intestinal transit characteristics, hormones, and corticosterone

levels is needed to clarify the mechanisms responsible for the corticosterone rhythmic differences shown here.

Finally, when 24-h rhythmic components were detected, all 3 rhythmic properties displayed more variability among the no-fiber than the high-fiber groups, as shown in Table 1. This is the first report of systematically varying 4 enteral nutritional options including fiber, and to describe such a finding. Because the 2 formulas were identical except for fiber content, a logical next step is to examine the influence of fiber on corticosterone temporal patterns. As mentioned above, gut peptides, other hormone substances such as insulin (Dallman and others 1995), and neural inputs have been linked with glucocorticoid release and levels. Proposed mechanisms can include direct stimulation of the adrenal cortex, or indirect stimulation through other routes, such as elements of the hypothalamus-pituitary-adrenal pathway. With the physiologic ileal brake acting to inhibit small intestinal motility, gut contents or speed or both may be essential not only to maintain normal corticosterone periods but also to stabilize other detectable rhythmic properties.

Conclusion

Although direct extrapolations cannot be made from animals to humans, these findings, nevertheless, suggest the possibility of rhythmic alterations and disruptions in the primary glucocorticoid with some enteral feeding options used in clinical practice. All but 1 group receiving enteral feedings on a 12-h schedule showed a loss of the well-established 24-h corticosterone rhythmic component. Additionally, in this small animal sample, when temporal patterns were present, or suggested, there was more variability in their rhythmic properties when no-fiber, rather than high-fiber, formula was used. The underlying mechanisms for such differences remain to be elucidated.

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