THE EFFECTS OF VASOPRESSIN ON PAIN PERCEPTION

By

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To my loving and supportive parents, Jeff and Marylee
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THE EFFECTS OF VASOPRESSIN ON PAIN PERCEPTION

By

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Major: Dental Sciences

Introduction: The neuropeptide vasopressin plays multiple important roles in the human body. Experimental evidence suggests vasopressin produces analgesic effects, though the findings have been mixed. Little research, however, has been focused on vasopressin’s modulation of inflammatory pain in human subjects, which may provide more clinically-relevant information. Methods: The study design was a randomized, placebo-controlled, crossover study. Through the standardized methods of Quantitative Sensory Testing, we examined the analgesic effects of a vasopressin analogue, desmopressin (DDAVP), compared to saline placebo in 40 healthy volunteers. Both non-inflammatory (heat, pressure) and inflammatory (capsaicin) pain models were assessed in two identical sessions, differing only in whether the subjects received desmopressin or the vehicle control. We also analyzed any sex differences in the analgesic properties of desmopressin. By using capsaicin in conjunction with Quantitative Sensory Testing, we were able to evaluate the effects of desmopressin on inflammatory and non-inflammatory experimental pain models. Results: No decreases in pain responses to inflammatory, heat, or pressure pain models with desmopressin were found when overall data was compared. However, a significant drug by order
interaction did occur with regards to inflammatory analgesia when desmopressin was
administered in the second session. No significant sex differences were found with
respects to desmopressin's analgesic properties. Conclusion: These data suggest that
vasopressin does play a role in the modulation of inflammatory pain, however, further
research is necessary to improve protocols regarding dosage and mode of
administration to enhance the effectiveness of vasopressin on pain responses.
CHAPTER 1
INTRODUCTION

Pain Overview

Pain, as described by the International Association of the Study of Pain, is an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage. Pain is a complex perceptual experience that can be expressed in a variety of forms (e.g., neuropathic, arthritic), regions (e.g., localized or general), and intensities (mild to severe). Given this complexity, the study and treatment of pain has been a challenge to the researcher as well as the practitioner. However, much advancement has been made in the field of pain research, opening up new avenues and approaches to studying this complex field.

In order to understand pain, one must gain an appreciation of the neuroanatomy and circuitry of the system. Nerve fibers found throughout the body convey information to the spinal cord and brainstem about a wide range of different stimuli. Nociceptive messages from the periphery are conveyed from primary afferent axons arising from somata of cells located primarily in the dorsal root ganglia in the body and trigeminal ganglia in the head. Impulses traveling along first-order neurons synapse on second-order neurons in the dorsal horn of the spinal cord or in the trigeminal nucleus complex in the brainstem. The neurons cross the midline to the contralateral side and ascend to synapse on third-order neurons, primarily in the thalamus. The main thalamic nuclei involved in relaying all somatosensory information to the cortex, including pain, are the ventral posterolateral nucleus (for the body) and the ventral posteromedial nucleus (for the head). The third-order neurons send fibers to the cortex where conscious awareness of the sensation occurs.
The main subtypes of primary afferent neurons can be divided into three major categories: A-β fibers, A-δ fibers, and C-fibers. A-β fibers are activated by innocuous touch, vibration, and joint pressure. A-δ fibers respond to intense and potentially dangerous mechanical or thermal stimuli, and C-fibers respond to a wide range of thermal, mechanical, and chemical stimuli. All three of these nerve fibers are involved in a variety of pain sensory pathways and processing functions. Signaling to and from these nociceptors occurs with the use of a variety of substances, including neuropeptides and chemokines. As an example, when nociceptors are activated by tissue injury, the neuropeptide, substance P, is released from vesicles at the axonal end of the nerve fiber. Substance P, in return, increases the rate of firing at the nociceptor via an autocrine and paracrine manner. In addition, cellular damage and inflammation increase concentrations of other chemical mediators such as histamine, bradykinin, and prostaglandins in the area. These additional mediators act synergistically to augment the transmission of nociceptive impulses along sensory afferent fibers.

**Vasopressin**

Another important neuropeptide involved in essential biological functions is vasopressin, also known as arginine vasopressin (AVP). AVP is mainly synthesized in the supraoptic nuclei and to a lesser extent the paraventricular nuclei of the hypothalamus. It is synthesized as a large prohormone and after packaging into secretory granules, the prohormones pass by axonal flow to the nerve terminals (Herring bodies) in the neurohypophysis. During this passage, the prohormones are cleaved into the biologically active AVP and a larger polypeptide fragment, neurophysin II. The neurophysin is then co-secreted with the active AVP upon electrical activation of
the neuro-secretory cells.\textsuperscript{4} Among the roles of AVP are hormone regulation, fluid regulation, cardiovascular regulation, body temperature regulation, and learning and memory influence.\textsuperscript{3,4,5} In the kidney, AVP acts on the G-protein linked Vasopressin 2 (V\textsubscript{2}) receptors on the capillary (basal) side of the distal convoluted and collecting ducts and stimulates the synthesis of cAMP.\textsuperscript{4} The cAMP activates a kinase on the luminal (apical) side of the ducts that initiates a series of events culminating in the insertion of water channels, known as aquaporins, into the luminal membrane.\textsuperscript{4} Along with the regulation of fluid intake, AVP also plays a role in cardiovascular activity in such that a reduction in effective blood volume leads to AVP release. AVP secretion is stimulated by a volume reduction of just 5–10\%.\textsuperscript{4} This is controlled by so-called stretch receptors that detect changes in blood volume/pressure.\textsuperscript{4} These include the baroreceptors and other receptors in the cardiovascular system.\textsuperscript{4} Figure 1 highlights these processes.

However, vasopressin has been shown to elicit effects in areas of the brain as well, leading to the involvement of the pain response in animal models.\textsuperscript{6} The periaqueductal gray seems to be an important area of the brain where vasopressin affects the nociceptive response. It was shown that pain stimulation lead to an increased secretion of arginine vasopressin in the hypothalamic paraventricular nucleus in the brain, leading to an increase in the periaqueductal gray.\textsuperscript{6} In an article by Yang, et al., the authors concluded that intraventricular injection of AVP increases pain threshold, and administration of anti-AVP serum decreases pain threshold.\textsuperscript{6} They found pain stimulation induced AVP secretion in the caudate nucleus. Their results also indicated that caudate nucleus pretreatment with an AVP-receptor antagonist reversed the effect of AVP by decreasing the pain threshold.\textsuperscript{6}
These results, in addition to another study by Yang, et al. involving AVP administration into the raphe magnus of rats, suggest that AVP plays an important role in antinociception. In another study using a rat model, it was observed that microinjection of AVP into the central nucleus of amygdala and to the nucleus of the spinal trigeminal tract produced potent antinociceptive effects, significantly inhibiting the jaw-opening reflex induced by tooth pulp stimulation in rats. The results of this experiment suggest that perfusion of rat cerebral ventricles with AVP has an inhibitory effect on the transmission of impulses from the sensory trigeminal neurons to the motor neurons in the hypoglossal nerve nucleus.

Despite the growing body of work from animal studies, there are very few reports of human testing using vasopressin and its various analogues in reference to pain. Pohl et al. used desmopressin as an analogue and tested its analgesic effects after nasal inhalation. In this study, thermal, mechanical, and ischemic pain stimuli were tested and the analgesic effects of desmopressin analyzed and assessed. Using two treatment doses of desmopressin, they found that the 30µg doses induced sensitization to thermal stimuli, and the mechanical pain threshold was increased by the 60µg dose. From this data it seems that desmopressin was effective for mechanical stimuli, but had the opposite effect for thermal. No effects were obtained for either dose on ischemic pain. While evoked experimental pain was evaluated in this study, a more clinically-relevant laboratory pain model, such as inflammatory pain was not tested in response to desmopressin. The limited research on the effects of vasopressin on a clinically-relevant human pain model provides an opportunity for additional analgesic studies.
Quantitative Sensory Testing

Quantitative sensory testing (QST) is a reliable method for recording and quantifying pain in humans. QST uses sophisticated neurophysiologic techniques to test both the nociceptive and non-nociceptive systems in the periphery and the central nervous system. It uses standardized mechanical and thermal stimuli (e.g., graded von Frey hairs, several pinprick stimuli, pressure algometers, quantitative thermotesting). Additionally, QST can assess and quantify sensory nerve function in a non-invasive fashion. QST is based on a precise definition of the stimulus properties (modality, intensity, spatial and temporal characteristics), analysis of the quality of evoked sensation and quantification of its intensity. In addition to assessment of sensory thresholds (i.e. detection threshold for innocuous stimuli and pain threshold), QST can include the assessment of sensations evoked by suprathreshold stimuli. The light-touch and vibration testing modalities evaluate the large myelinated A-α and A-β sensory fibers, whereas the thermal testing modality assesses small myelinated and unmyelinated sensory nerve function. Using QST, one can study the function of large and small myelinated, and unmyelinated fibers, in addition to documenting hyperalgesia and hypoaesthesia. QST can allow an investigator to assess a loss of function (minus signs) as well as a gain of function (positive signs). Allodynia or hyperalgesia, for example, can be quantified by measuring intensity, threshold for elicitation, duration and area.

However, just as in any other testing modality, standardization of instructions to subjects, training of technicians, machine calibration, stimulus characteristics, and testing algorithms are all necessary for accurate and reproducible results. One previous
complication with the use of QST devices for sensory thermal testing has been limitations due to large inter-individual variations including unreasonably low thresholds for thermal pain, lack of data on intra-individual variations over time, and on the subjects’ perception at threshold. In a study by Wasner and Brock, they demonstrated that previous experience of test stimuli has no influence on the variability of thermal pain thresholds, reproducibility of determinations of heat and cold pain thresholds within individuals, or the potential value of pain and temperature ratings at the thermal pain thresholds.\textsuperscript{13} Their results concluded that measurement of thermal pain thresholds showed good reproducibility over time.

**Capsaicin**

One concern regarding typical stimuli used during QST has been their potential lack of clinical relevance. However, experimental pain models, such as those using topical capsaicin for both algesic and analgesic effects, are available that more closely mimic some forms of clinical pain. Capsaicin is the primary active component of the heat and pain-eliciting lipid-soluble fraction of the Capsicum pepper and was first isolated in crystalline form by P. A. Bucholz in 1816. Capsaicin is practically insoluble in water, but freely soluble in alcohol, ether, benzene and chloroform.\textsuperscript{14} Its clinical application has been intensely studied over the years, especially concerning its role in pain modulation. In animals and humans, recordings from single sensory afferents have shown that A-delta- and polymodal C-fibers respond to capsaicin application.\textsuperscript{15} The pain evoked by capsaicin is highly dependent on the application method (intradermal, intramuscular, topical). Using topical capsaicin, a high degree of spatial summation of impulses from the activated nociceptors contributes to the pain sensation.
Thus, topical capsaicin application represents a tonic nociceptive input, and it is used in many experimental pain models as a conditioning stimulus.\textsuperscript{15}

Capsaicin is an agonist of the transient receptor potential channel vanilloid 1 (TRPV1) channel, also known as the vanilloid or capsaicin receptor. The receptor was cloned in 1997, and it is considered to act as an integrator of various physical and chemical nociceptive stimuli, as it can be gated by noxious heat (\textgreater 43°C), low pH and also by recently described endogenous lipids.\textsuperscript{16,17,18} Initially, TRPV1 was only thought to exist in small to medium diameter neurons and only in the dorsal root, trigeminal, and nodose ganglia.\textsuperscript{19} Recent studies, however, have demonstrated that TRPV1 is expressed in many other neuronal and nonneuronal locations, although the expression level in small diameter peripheral sensory neurons appears to be at least 30-fold higher than any other location in the body.\textsuperscript{19}

Clinically, it has been noted that application of capsaicin can result in both analgesic and algesic properties, depending on the dose, concentration, and route of administration.\textsuperscript{20,18} For example, neurotoxicity and selective degeneration of capsaicin sensitive primary neurons occurs following systemic high concentration capsaicin administration in neonatal rats.\textsuperscript{21,18} Also, high concentration intradermal injection of capsaicin was shown to produce degeneration of epidermal nerve fibers and reduced pain sensitivity in humans.\textsuperscript{22}

The application of capsaicin can have many experimental benefits in animal and human pain trials as an inducer of neurogenic-inflammatory pain. One study demonstrated that intradermal application of low concentration capsaicin leads to acute pain, followed by mechanical hyperalgesia in humans and to sensitization of
In another study to characterize the role of capsaicin-sensitive primary afferents in inflammatory pain, capsaicin was used to elicit inflammatory symptoms. Chen et al. used different methods of capsaicin application then assessed the outcomes of pain perception using four different animal models of inflammatory pain. The nociceptive responses of the rats have demonstrated that capsaicin-sensitive primary afferents play differential roles in persistent spontaneous nociception, thermal and mechanical hyperalgesia, and inflammation in different inflammatory pain models. Therefore, the use of capsaicin as a pain modulator in pain studies has great applicability since a capsaicin-induced pain response provides a clinically relevant inflammatory model for evaluating nociceptive alterations.

**Purpose and Hypothesis**

Limited human research has been performed concerning the role of vasopressin in pain modulation, specifically its function as a suppressant of inflammatory pain. With this in mind, we intend to further characterize the role of vasopressin in pain processing with the goal of gaining a better understanding of human pain perception. By using capsaicin in conjunction with more traditional QST measures, we can evaluate the effects of vasopressin on inflammatory and non-inflammatory experimental pain models and strive for the ongoing progression in the better treatment of pain conditions. The overall intent of this research is to determine the analgesic effects of desmopressin in humans using laboratory pain assessment methods. We hypothesize that desmopressin, a synthetic analogue of vasopressin, will significantly decrease experimental and neurogenic inflammatory-induced pain, as compared to the vehicle-control.
Figure 1-1. (Den and Meinders 2005) Sites of action of vasopressin
CHAPTER 2
MATERIALS AND METHODS

Participants

We included healthy adults between the ages of 18 and 55. We excluded any participants with pain-related disorders or systemic medical conditions (e.g. diabetes, hypertension), psychiatric illness, pregnancy, and regular use of prescription pain medication. Participants were also instructed to not take any over-the-counter medications within 48 hours of testing sessions. Upon approval by the Institutional Review Board (IRB), we enrolled a total of 40 subjects for this protocol, with approximately equal numbers of males and females. Subjects were recruited using posted advertisements and by contacting subjects who have participated in one of our other protocols and given us permission to contact them. When participants contacted our laboratory, we conducted a brief telephone screening to ensure that they met criteria for study inclusion. Upon selection, verbal and written informed consents were obtained by a trained research assistant. Next, the participant completed several questionnaires regarding their health history, pain coping, and psychological status and was then appointed for their two sensory testing sessions.

Experimental Design

Before each sensory testing session, participants completed a Visual Analog Mood Scale (VAMS), following which blood pressure and heart rate were measured using an automated blood pressure cuff assembly. Participants also completed a VAMS after administration of the test drug. Blood pressure was recorded periodically throughout the testing sessions and before dismissal of the participant. A biological sample was taken for DNA extraction and future genetic testing in order to identify
genes that may be associated with pain response. The biological sample was taken in the form of a saliva sample and collected in prepared, sterilized tubes.

After the above information was collected qualified participants completed two sensory testing sessions. The study was performed as a double-blind, vehicle-controlled crossover study. We assessed responses to thermal, pressure, and topical capsaicin stimuli before and after intranasal administration of the study drug. The sessions were separated by at least one week and by no more than 14 days. The two experimental drug sessions were identical, except that desmopressin was administered during one session and saline during the other, in randomized order, as depicted below. The order of administration of either desmopressin or saline was double-blinded and determined by the pharmacist. The participant was assigned either an even or odd number upon agreement to participate in the study. This number determined whether the patient received the heat test or pressure test first, and whether the masseter or the trapezius was pressure tested first. For example, an even numbered patient began with pressure pain test starting with the trapezius then masseter. The heat test then followed and the same order was kept through both sessions. Table 2-1 outlines the timing of the experimental events.

Thermal pain was assessed using a commercially available thermal simulator (Medoc, Ramat-Yishai, Israel) used widely in clinical settings. This device delivers thermal stimuli to the skin and is controlled by investigator inputs of computer software. Two types of thermal stimuli were delivered:

**Pain threshold/tolerance**: Slowly increasing thermal stimuli that the subject terminates by pressing a button when it reaches a pre-specified level. The Threshold
test included 3 trials and the patient was instructed to press the button when he/she first felt pain. The Tolerance test included 3 trials in which the participant pressed the button when the increasing pain was no longer tolerable. Both threshold and tolerance tests were done on the left arm and only threshold test was done on the right arm. Ratings for the Threshold/Tolerance portion were recorded as temperature readings displayed on the computer screen after the participant stopped the heating mechanism. A cut-off of 52° C was used to prevent burning or tissue damage.

15-Second heat stimuli rating (for first 20 participants only): For a brief period (15 seconds) heat pulses were administered and participants asked to rate their pain at 5, 10, and 15 seconds. Three trials were done each with increasing temperatures of 47°, 48°, and 49° C. Participants were also asked to rate their pain 15 and 30 seconds after the last heat pulse. This heat test was done only on the left arm. Pain ratings for the 15-second stimulus were obtained using standardized 0 to 100 numerical visual analog scale (VAS), where 0 was no pain and 100 denoted the most intense pain imaginable. NOTE: This section was eliminated during testing of participants 21-40.

Pressure pain was assessed using a handheld algometer with a 1-cm diameter tip. Pressure stimuli were applied to the trapezius muscle and the masseter muscle. Pressure was slowly increased until the participant indicated pain threshold, at which time the algometer was removed and recordings taken. The recordings are numerical values displayed on the handheld algometer in units of kg/cm². A minimum of 3 trials were performed at each site until two consistent readings were obtained. Pressure pain stimuli was only applied to the left trapezius and left masseter.
**Touch sensitivity:** Dynamic mechanical allodynia was assessed using a cotton swab stroked (1 cm/sec) across the site of capsaicin application at a pressure sufficient to slightly bend the stem of the cotton swab. This was repeated 3 times, and the subjects were asked to provide a pain rating of yes or no, signifying whether they felt pain or not with the cotton swab swipe.

**Application of Capsaicin:** The inflammatory pain stimulus for this proposal is capsaicin. Capsaicin is available as an over the counter cream (0.025%, 0.075%) for topical treatment of pain in patients suffering from arthritis and various neuralgias. For this study, we used an 8% capsaicin transdermal cream. This concentration of capsaicin produces moderate but tolerable pain in the vast majority of participants, and there have been no significant adverse reactions. Capsaicin was supplied by Formosa Laboratories (Taoyuan, Taiwan), with the final 8% transdermal cream manufactured by a local compounding pharmacist. The cream was applied to a 3 cm$^2$ area overlying the ventral right forearm. Participants were instructed not to touch this area during the course of the study to minimize spreading of the cream to other areas of the body. At the end of the session, the subjects’ arm was wiped clean with alcohol swabs then with gauze or a towel soaked in dairy cream to remove residual capsaicin.

Desmopressin, 50 µg of DDAVP (Sanofi Aventis US LLC), was delivered intranasally. Intranasal desmopressin (DDAVP Rhinal Tube) at a concentration of 100 µg/ml was administered using a Valois VP7 metered spray pump. The DDAVP was self-administered as a series of five 100 µl sprays, for a total dose of 50 µg. Desmopressin nasal solution was transferred by the investigational pharmacy from the original manufacturer’s packaging into sterile nasal pumps using aseptic technique in
certified Class 7 ISO clean room. This preparation occurred less than 30 minutes prior to drug administration to minimize drug degradation. The vehicle used was Phosphate-Buffered Saline (PBS) and it was delivered in the same fashion as the desmopressin (e.g., 5 x 100µl sprays).

**Statistical Analysis**

Pain responses were compared after desmopressin to pain responses after saline. These drug effects were analyzed using separate 2 (drug) X 2 (gender) mixed model analyses of variance (ANOVAs) for each of the three pain measures (capsaicin, thermal, and pressure). Because previous human research with desmopressin and pain is limited, sample size was determined in order to power the experiment to detect moderate effect sizes for both the drug effects and the sex difference, after accounting for the possibility of some attrition or missing data.
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<td>15-25 minutes</td>
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<tr>
<td>120 minutes</td>
<td>Dismiss</td>
<td>Blood pressure &amp; heart rate until baseline value</td>
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CHAPTER 3
RESULTS

General Data

A total of 40 participants were enrolled in the study with equal numbers of males and females. The average ages of the males and females are demonstrated in Figure 3-1. The ages of the males ranged from 20 to 39 years old and the ages of the females ranged from 19 to 28 years old. Participants came in for 2 sessions of 120 minutes each. For the first part of the session, measurements were taken for heat and pressure pain thresholds. These tests were done before vasopressin, placebo, or capsaicin was administered. This allowed for a baseline measurement of each participant, independent of any drug or placebo effects. The second set of tests was done after either vasopressin or vehicle was given to the participant. This allowed for results relating directly to vasopressin’s role on heat and pressure stimuli. Finally, the third set of measurements was taken after drug/placebo administration, and also after application of capsaicin. The effects of vasopressin on clinically relevant inflammatory pain were derived from this data set, which was the main focus for the aims of this study.

Figures 3-2 through 3-5 show the overall effects of desmopressin on pressure, heat, and inflammatory pain. Figure 3-2 shows desmopressin’s effect on pressure pain thresholds for both the masseter and the trapezius muscles. The red and blue bars distinguish between desmopressin and saline placebo ratings and are broken down into pre-drug and post-drug. The scores are in kilogram units recorded from the algometer when the participant reached their pain threshold. As seen from the bar graphs, no statistically significant differences were noted for drug versus the saline placebo.
Figure 3-3 depicts heat pain thresholds for the left arm before and after drug or placebo was administered. The left arm was the non-capsaicin site and simply represents the effects of desmopressin on heat pain. The three points on the graph, Pre-Drug, Post-Drug1, and Post-Drug2 correlate with threshold temperature readings taken before drug/placebo was administered, 10 minutes after drug/placebo was administered, and 45 minutes after drug/placebo was administered, respectively. Again, no statistically significant drug effects were detected at any of the time intervals.

Figure 3-4 is a similar plot, except the points on this graph represent threshold temperature ratings taken from the right arm where the capsaicin was administered and signify heat hyperalgesia effects of desmopressin. The three time intervals, Pre-Cap, Post-Cap1, and Post-Cap2 correlate with temperature ratings taken after drug/placebo was administered but before capsaicin was applied, 10 minutes after capsaicin application, and 45 minutes after capsaicin application, respectively. The temperatures correspond with the threshold point at which the participant stopped the heat application to the right arm. No statistically significant differences were noted for desmopressin and placebo threshold ratings.

Figure 3-5 is a plot representing desmopressin’s effects solely on capsaicin pain. During this part of the session, the participants were asked to rate their pain relating only to the capsaicin site on a scale from 0-100. This took place over a 50-minute time period where they made ratings every 10 minutes. As can be seen, no statistically significant drug effect on capsaicin-related pain was observed.

**Session Data and Sex Differences**

The next two plots break down pain threshold temperatures and pain ratings based on whether desmopressin was administered in session 1 or session 2 (i.e., to
examine order effects). In Figure 3-6, the left side of the plot shows data for subjects in whom desmopressin was administered in session 1 and the right side of the plot shows the group that received desmopressin in session 2. The blue and red colors show the desmopressin and saline placebo ratings, respectively. As before, the Pre-Cap, Post-Cap1, and Post-Cap2 time intervals represent pain threshold temperatures before capsaicin application but after drug/placebo administration, 10 minutes after capsaicin application, and 45 minutes after capsaicin application, respectively. Since these temperature thresholds were taken from the right arm at the capsaicin site, they represent any effects of desmopressin on capsaicin-induced heat hyperalgesia. As can be seen from Figure 3-6, when desmopressin was administered in session 2, there is a significant difference in the threshold ratings compared to vehicle, resulting in a significant drug by order interaction.

Figure 3-7 depicts capsaicin pain ratings, also split into whether desmopressin was administered in session 1 or session 2. The pain ratings are on a 0-100 scale and represent desmopressin’s effect on capsaicin pain. Again, as seen when desmopressin was administered in session 2, the pain ratings were significantly lower than the vehicle pain ratings. This resulted in a significant drug by order interaction. Thus, Figures 3.6 and 3.7 indicate that desmopressin produced significant analgesia against capsaicin-related pain, but only among subjects who received desmopressin in session 2.

Figures 3-8 and 3-9 show data which corresponds to one of the other aims of this study: sex differences with respect to the effect of desmopressin. In Figure 3-8, pressure threshold ratings were compared between males and females with both pre-drug and post-drug effects. As can be seen, desmopressin did not have a significant
effect when it came to differences between males and females. There was, however, a significant sex difference in pressure pain thresholds, both pre-drug and post-drug. It can be seen that the males consistently had higher thresholds to pressure pain compared to females.

In Figure 3-9, males and females were compared with respect to their ratings for capsaicin pain. These pain ratings were taken after capsaicin application on the right forearm and show 10 minute interval means for 50 minutes after capsaicin application. When the ratings are compared, there is no sex difference between males and females. However, when minutes 40-50 are reached, the pain ratings for males start to decrease, whereas the pain ratings for females continue to increase. This results in a significant sex by time interaction between males and females.
Figure 3-1. Average age and participant ethnicity

Figure 3-2. Effect of desmopressin on pressure pain thresholds. No effect of drug \[F(1,37)=2.11, p=0.155]\]
Figure 3-3. Effect of desmopressin on heat pain thresholds on the left arm (non-capsaicin site). No effect of drug \([F(1,37)=0.38, p=0.541]\])

Figure 3-4. Effect of desmopressin on heat pain thresholds at the capsaicin site (heat hyperalgesia). No effect of drug \([F(1,37)=2.47, p=0.124]\])
Figure 3-5. Effect of desmopressin on capsaicin pain. No effect of drug \( [F(1,37)=0.20, \ p=0.66] \)

Figure 3-6. Influence of drug order on desmopressin. Effects on heat pain thresholds at the capsaicin site. Significant Drug X Order interaction \( [F(1,37)=19.49, \ p < 0.0001] \)
Figure 3.7. Influence of drug order on desmopressin effects on capsaicin pain. Significant drug X order interaction \( [F(1,36)=15.83, p=0.0003] \)

Figure 3.8. Sex differences in pressure pain thresholds. Significant sex difference - Masseter \( [F(1,37)=8.88, p=0.005] \) - Trapezius \( [F(1,37)=11.00, p=0.002] \)
Figure 3-9. Sex differences in capsaicin pain. No overall sex difference, but a significant sex X time interaction [F(4,144)=3.02, p=0.049]
CHAPTER 4
DISCUSSION

General Data Analysis

The role of vasopressin as a pain modulator has only been superficially explored in human subjects. AVP’s known effects in cardiovascular, metabolic, and regulatory pathways are well documented, however, and provide initial evidence as to the importance of this hormone’s regulatory role in the human body. The aims of this study were to evaluate the role of desmopressin on clinically relevant inflammatory pain and to analyze the potential effects of dose and gender on pain outcomes.

The cross-over study design allowed for complete randomization of the participants into two groups over two separate sessions: those that received the active drug, desmopressin, first versus those that received the saline vehicle first. Since this was also a double-blinded study, neither the researchers nor the participants knew what form of nasal inhalant they were receiving. Each session took pre-drug/placebo pain ratings, post-drug/placebo but pre-capsaicin ratings, and post-drug/placebo, post-capsaicin ratings. This not only allowed for a baseline pain evaluation during each session, but also granted application of sessional statistical analysis. In addition to being able to examine the overall effects of desmopressin among participants, we were able to look at consistencies or conflictions on a session by session basis.

Figures 3-6 give an overview of the general responses from the participants to pain stimuli derived from both the drug and placebo. As can be seen from the pressure ratings on the masseter and trapezius in Figure 3-2, desmopressin had no analgesic effect compared to vehicle. Contradictory results were found in the Pohl et al. study where pain thresholds and tolerances were tested through mechanical stimulation on
the fingers.\textsuperscript{9} They used two different doses of desmopressin, 30µg and 60µg, both of which significantly enhanced pain tolerance. Our desmopressin dose was 50 µg, and their mechanical stimuli involved more of a finger prick compared to our pressure algometer. These two different methods of protocol allowed for some variability when comparing the studies and the results and might explain the different outcomes that were generated.

Our thermal stimuli results, however, were consistent with those found in the Pohl et al. study. They concluded that desmopressin had no significant effect, at either dose, on thermal pain.\textsuperscript{9} Although their method of heat delivery was slightly different, similar outcomes were found with our thermal nociceptive stimuli, represented by Figure 3-3. No statistically significant differences were found in the temperature threshold ratings between those participants that received vasopressin and those that received placebo. These outcomes, along with our pressure stimuli results, suggest a limited role in pain modulation of vasopressin for this group of participants for both pressure and heat stimuli.

The main aim of this study was to analyze the effects of vasopressin on inflammatory pain. We attempted to best mimic inflammatory pain in a clinically relevant manner by using a capsaicin cream. The cream elicited a superficial inflammatory reaction on the skin of the right forearm at which thermal and capsaicin related pain were recorded. Figures 3-4 and 3-5 represent the overall data associated with capsaicin related pain. Figure 3-4 shows threshold temperatures taken at the capsaicin site before application, as well as 10 and 30 minutes after application of capsaicin. Capsaicin produced a significant decrease in thermal threshold, but no statistically significant drug
effects were found. Figure 3-5 has pain ratings associated with the capsaicin site for 10-50 minutes after application. And although there was an increase in pain ratings over the time period, they were consistent across drug conditions with no significant differences.

**Session Data Analysis**

These overall data are limited, however, in their interpretation, as they do not address the effect of drug order. Our cross-over study design involved two almost identical sessions, differing only in what solution they received in the nasal inhalant, and despite randomization to drug order, order effects emerged. When the data were examined further, it was revealed that significant desmopressin analgesia was present among subjects who received desmopressin during session 2, but not for those who received desmopressin in session 1. This pattern emerged both for capsaicin pain and for measures of capsaicin-induced heat hyperalgesia.

It becomes difficult to assess whether these statistically significant differences can be applicable in a clinically relevant manner. The study design allows for a session effect to occur, and this must be evaluated and anticipated in any randomized cross-over study. From the data expressed in the graphs in Figures 3-6 and 3-7, it can be noted that when desmopressin was given in session 1, the numbers indicate that vehicle has a slightly more analgesic effect than desmopressin. Although these effects were not statistically significant, it warrants further attention.

There is always the possibility of a participant’s anticipation and reporting of pain being affected by an increase in comfort associated with the familiarity of a second session. Moulin et al. disclosed similar findings in their randomized crossover trial of morphine and placebo for chronic pain patients. They reported a sequence effect, with
their participants having similar pain intensity outcomes for morphine and benztropine placebo depending on which order the drug was administered. In our study, these session effects can be downplayed since we had significant outcomes for desmopressin when it was administered in session 2. However, in the Moulin et al. study, their order effects dictated that morphine was superior to placebo in producing analgesia only during the first crossover period. So the anticipation of this phenomenon, which even exists in carefully conducted clinical trials with well documented opioid analgesics such as morphine, must be considered when interpreting results.

At this time, we are unable to provide an explanation for the order effect that occurred in the present study. One interpretation is that participants had a higher level of anxiety in session 1 leading to a disturbance that might have affected the analgesic properties of desmopressin. Another theory is the possibility that expectancies for desmopressin analgesia may have been greater in session 2 than in session 1. Either way, it then becomes possible that the analgesic effects of desmopressin might have been partially masked during this study, rendering further investigation necessary.

**Sex Difference Analysis**

Another aim of this study was to assess any sex differences within the effects of vasopressin’s analgesic properties. There are a wide range of studies within the literatures discerning differences in males and females with regards to their pain perceptions, tolerances, and reactions to painful stimuli. In a study by A. Dawson and T. List, electrical and pressure stimuli were applied and evaluated over a group of Middle Eastern and Swedish men and women. They found significantly different pain tolerance levels for both electrical and pressure stimuli between men and women, with men having a significantly higher tolerance. These results paralleled well with an older study
by Feine and Bushnell, et. al. This study only examined noxious heat stimuli, but they found that women rated noxious heat stimuli as more intense than males did.27

In Figure 3-8 the pressure related data with respect to males and females is presented. As seen from the graphs, although no significant sex differences were found with the analgesic effect of vasopressin, an obvious difference in tolerance levels between males and females does exist. Males have significantly higher pain tolerances to pressure stimuli on both the masseter and trapezius compared to females. This data supports conclusions drawn from the A. Dawson and T. List article where they found similar results with their pressure stimuli recordings.

In Figure 3-9 of the present study, capsaicin pain was compared between males and females across time for vasopressin and placebo. It was found that a significant effect of desmopressin did not result when men and women were compared. Pain ratings essentially remained constant up to about 40 minutes after capsaicin application. However, a significant sex by time interaction did develop after 40 minutes for the saline portion of the experiment. This can be seen from the graphs in Figure 3-8. When the females were given the saline placebo, their pain ratings increased significantly more than the males who were given placebo after 40 minutes. This resulted in a significant sex by time interaction. It is difficult at this point, without further investigation, to decipher any significance related to vasopressin or capsaicin with respects to these outcomes, but hopefully it might provide some insight as to what might be expected with continued research.

**Critiques**

One of the main critiques we had for our study was the fact that there was a distinct taste difference between the saline placebo and desmopressin nasal inhalants.
This was noted on occasion by multiple participants. It is unlikely that the participants were able to correctly determine the nature of the nasal inhalant, even if they could decipher a difference in taste between the two nasal solutions. But it is possible that their internal impression of what the solution was could have affected their pain ratings on some level. This was an inherent insufficiency of our study, and future vasopressin research with a desmopressin nasal vehicle should design a drug and vehicle with identical taste properties.

Another improvement that could have been made was related to the capsaicin experience of the participants. It would have been beneficial to have a baseline capsaicin appointment to reduce novelty effects with the capsaicin cream. For the participants, this was a new and unique feeling, and in a few instances we noticed they had difficulty defining the sensation they were feeling on their forearm. Responses ranging from tingling, itching, numbness, and extreme burning were reported by various participants. However, not only was it difficult for them to distinguish this as pain, but it was also hard for them to extrapolate a painful 0-100 scale rating for the sensation they were encountering. This was not as much a problem in session 2 as it was in session 1 where the unfamiliarity resided. This is why an introductory capsaicin session might have improved the participants' interpretation of the inflammatory pain they were experiencing.

**Conclusion**

This study found some evidence that vasopressin has a regulatory role with regards to modulation of nociceptive stimuli. Although the level of significance needs to be further explored, this role is probably more pronounced with regards to inflammatory stimuli than with any other nociceptive related stimuli. Concomitant investigation is
necessary to explore alternative doses and modes of administration of vasopressin so as to test its analgesic properties, especially with inflammatory pain. Instrumental to future studies will be the need to alleviate any session effect that was seen in the present study. Also, any additional studies should make every attempt to eliminate the insufficiencies that presented during this study, including any taste discrepancies between the drug and placebo.
LIST OF REFERENCES


BIOGRAPHICAL SKETCH

Jeffrey Bradford Mokris was born in January 1981 in Charlotte, North Carolina to Jeffrey G. and Marylee Mokris. Brad lived in Charlotte with his three younger brothers until 1999 when he graduated from Charlotte Catholic High School. He went on to attend The University of North Carolina at Chapel Hill where he earned his Bachelor of Science in biology with a minor in chemistry. After working outside of Honolulu, Hawaii for a year, he started his dental education at the University of Florida College of Dentistry where he received his Doctorate in Dental Medicine in 2008. After graduation, Brad immediately began his residency in orthodontics and dentofacial orthopedics. In May of 2011, he graduated with a Master of Science degree and a Certificate of Orthodontics.