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The Rat as a model of female sexual arousal.

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Abstract

Female Sexual Dysfunction (FSD) is a significant problem affecting 25% - 63% women in the U.S. alone. Although the research community has begun to increase efforts to understand FSD, there is a lack of an animal model in which this condition can be studied. The current research provides data for a new model of sexual arousal in the female Sprague-Dawley rat. The vascular effects in the clitoris and vagina in response to clitoral and pelvic plexus nerve stimulations were studied. Modulation of these effects by infusion of Nitric Oxide, Vasoactive Intestinal Peptide and Calcitonin-Gene Related Peptide agonists and antagonist in the clitoris was also observed. Histological sections of the clitoris were examined for the endogenous presence of these neurotransmitters. These data provide the framework for a reproducible model of sexual arousal in the female rat.

Abrégé

La dysfonction sexuelle féminine est un problème important affectant entre 25%-63% des femmes aux États-Unis. La communauté scientifique a récemment intensifié ses efforts à mieux comprendre ce désordre. Un manque de modèles animaux permettant l'étude de cette condition a limité cependant leur recherche. L'étude actuelle présente des donnés sur un nouveau modèle d'excitation sexuelle chez le rat femelle Sprague-Dawley. Les effets vasculaires du clitoris et du vagin en réponse aux stimulations électriques des nerfs du clitoris et du plexus pelvien ont été étudiés. La modulation de ses effets par l'infusion d'agonistes et d'antagonistes du monoxide d'azote (Nitric Oxide), du Vasoactive Intestinal Peptide et du Calcitonin-Gene Related Peptide dans le clitoris a également été observée. Des sections histologiques du clitoris ont été examinées afin d'identifier la présence de ces neurotransmetteurs. Ces résultats donnent un cadre pour un modèle reproductible de l'excitation sexuelle chez le rat femelle.

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Contributions of Authors

In the article titled Increases in clitoral and vaginal blood flow following clitoral and pelvic plexus nerve stimulations in the female rat: Dr.'s Pascal Vachon and Serge Carrier were responsible for generating the ideas methodology used to perform the experiments. Neil Simmerman was responsible for performing the experiments, data analysis, preparing the background research and writing the manuscript under the guidance of Dr.'s Carrier and Vachon. Dr. Abdel Zahran served as a consultant to the experimental protocols and background research.

In the article titled Female Sexual Dysfunction: A Vasculogenic Model In The Female Rat: Neil Simmerman designed the experimental methodology and protocol described in this paper under the guidance of Dr.'s Carrier and Vachon. Neil Simmerman was responsible for performing all experiments and data analysis. The manuscript was written and prepared by Neil Simmerman.

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1.0 Introduction to Female Sexual Dysfunction.

Female sexual dysfunction (FSD) is an age related, progressive and prevalent problem that can affect the quality of life for many women. 25% - 63% of women in the U.S. suffer from some type of sexual dysfunction (1,2). Sexual dysfunction in women usually presents as Female Sexual Arousal Disorder (FSAD), Female Orgasmic Disorder (FOD) or Vaginismus (3).

The study of FSD is gradually evolving from simple extrapolations of studies from male erectile and sexual dysfunction into proper scientific research using methods specific to the study of females. Recently there has been a resurgence in FSD related research thanks in part to new technologies and analytical methods. New societies are been formed and international meetings are being held with female sexual dysfunction as the main agenda.

1.1 What is Female Sexual Dysfunction?

According to the DSM-IV there are 4 main categories of sexual disorder: Sexual desire disorders, Sexual arousal disorders, Orgasm disorders and Sexual pain disorders.

Sexual desire disorders include hypoactive sexual disorder and sexual aversion disorder. Hypoactive sexual desire is defined in the DSM-IV as persistent absence of sexual fantasy and desire for sexual activity that causes personal distress. It is also sub-classified with sexual

aversion disorder, which is the persistent extreme aversion to any genital contact with a sexual partner that causes personal distress (4). Hypoactive sexual disorder may be caused by a host of factors ranging from psychological/emotional to physiological dysfunction. Any disruption to the normal female hormonal state such as menopause, surgical intervention or endocrine disorder can result in decreased sexual drive (2). Sexual aversion disorder is more commonly a psychological disorder.

Sexual arousal disorders are the persistent failure to attain or maintain the lubrication-swelling response of sexual excitement until completion of the sexual activity (4). Arousal disorders are usually seen as a lack of vaginal lubrication, decreased clitoral and labial sensation and engorgement or lack of vaginal smooth muscle relaxation. Although these conditions can be the result of psychological factors there is usually a medical basis such as diminished blood flow, pelvic trauma or surgery (2).

Orgasm disorders are persistent delays in orgasm following a normal sexual excitement phase judged to be adequate in focus, intensity and duration. Primary anorgasmia can be secondary to emotional trauma. However, medical factors may certainly contribute to the problem (4).

Sexual pain disorders include dyspareunia and vaginismus. Dyspareunia is a recurrent genital pain before,

during or after intercourse. It can be either psychologically or physiologically based as a result of vestibulitis, vaginal atrophy or vaginal infection. Vaginismus is an involuntary spasm of the musculature of the distal third of the vagina that interferes with coitus. This spasm usually develops as a response to painful penetration or psychological/emotional trauma (2-4).

1.2 Incidence of Female Sexual Dysfunction in the U.S.

Despite the fact that FAD has only recently entered the public limelight, it is not a novel disorder. The U.S. National Health and Life Survey reported that 43% of women experience some form of sexual dysfunction (3). In addition, U.S. census information reports that 9.7 million women aged 50-74 self report complaints of diminished vaginal lubrication, pain and discomfort during intercourse, decreased arousal and difficulty achieving orgasm (3). This clearly infers that FSD is a relevant and pressing public issue. To date there are relatively few published articles cataloguing the incidence of sexual dysfunction among women (1,3,5). In addition, the epidemiology of FSD is also poorly understood. There are very few reliable estimates from population-based samples. Little is known at this time about the risk factors for FSD or the progression of the disease over a lifetime.

In order to properly understand FSD, a formal explanation of the anatomy and physiology of the female urogenital system and sexual response is first in order. With this knowledge, one can then delve into the pathophysiology of female sexual dysfunction.

2.0 Female Sexual Anatomy

The female external genitalia, or the vulva as it is commonly referred to, includes the mons veneris, the labia majora and minora, the clitoris, the hymen, the vestibule and the entrance to the vagina. The tissues of the vulva are heavily vascularized. They are supplied by branches of the internal pudendal artery and drained by both the internal pudendal vein and the saphenous vein (6,7).

2.0.1 Mons Veneris and Labia Majora

The mons veneris is the portion of the vulva that lies above the upper part of the symphysis pubis and the lower abdominal muscles. It consists of the hair bearing skin covering the fat deposits located above the pubic area in the female. Extending downwards from the mons are the labia majora. They consist of folds of skin above underlying fat and are homologous to the scrotum of the male. While the outer aspect of the labia majora contains hair follicles, the inner part is smooth and kept moist by the secretions of sebaceous and other glands. The labia majora come together to close the entrance to the vagina. The mons and labia majora are covered with rough skin, hair, sebaceous and sweat glands in addition to specialized apocrine glands whose secretions give rise to a characteristic odor, which is often of sexual significance (6,7).

2.0.2 Labia Minora

The labia minora are soft flaps of skin found within the labia majora and are homologous to the floor of penile urethra in the male. Anteriorly the labia minora join together in order to provide the clitoris with a prepuce and frenulum. Posteriorly they merge into the labia majora. The labia minora are extensively vascularized and often swell and become turgid during periods of sexual arousal (6,7).

2.0.3 The Clitoris

The clitoris is the female homologue to the penis. It is derived embryonically from the genital tubercle. It is located on the front of the symphysis pubis, and is usually covered by the labia majora. Like the penis it contains a glans, prepuce, body and two crura which attach it to the pubic bones. However, only the glans and prepuce are visible. The clitoris itself is 2-4cm long while the crura average 10cm in length. The clitoris is an erectile organ comprised mainly of erectile tissue with a generous nerve supply, the latter making it the most sexually sensitive part of the female genitalia. The corporal bodies of the clitoris become engorged with blood upon stimulation (2,6-8).

2.0.4 The Hymen

The hymen is a thin, avascular membrane that guards the entrance to the vagina. It contains at least one aperture for the outflow of menstrual blood. It is often torn during coitus and is a common sign for the loss of virginity. However, operations or insertion of tampons may also cause injuries. The hymen is destroyed during childbirth (6,7).

2.0.5 The Vestibule

The vestibule is the anatomical term used to describe the smooth skin within the labia minora in front of the vaginal opening and containing the urethral opening and the urethral labia. Beneath the vestibule lie the vestibular bulbs. These are a collection of erectile tissue homologous to the corpus spongiosum in the male. In the posterior portion of each vestibular bulb are the Bartholin's glands. These glands, analogous to the Cowper's gland in males, are oval in shape and pea sized. The duct of this gland runs downward and opens below the hymen. The secretion of the Bartholin's gland is colorless and mucoid with a characteristic odor. It is produced in response to sexual excitation and is utilized as a lubricant for coitus (6,7).

2.0.6 The Vagina

The vagina is an elastic fibrous and muscular walled canal extending backward from the vulva at an angle of 60-70

degrees above horizontal. The vagina pierces both the triangular ligament and the pelvic diaphragm and leads to the cervix. The vault of the vagina is divided into four areas relative to the cervix; the posterior fornix, which is the most capacious area of the vagina, the anterior fornix and the two lateral fornices. There is a raised double column formed by underlying fascia running sagitally down the anterior wall located on these columns are rugae which account for the distensibility of the vagina during childbirth. The vagina is lined by stratified squamous epithelium that is devoid of keratin but has the ability to become keratinized. The mucosa is not secreted solely from the lining of the vagina. It also consists of tissue fluids, epithelial debris, electrolytes, proteins and lactic acid with an average pH of 4.5. The epithelium rests on a connective layer made of elastic tissue covered by criss-cross layers of longitudinal and circular muscle layers. The muscles of the vaginal wall are involuntary (2,6,7).

2.1 Changes In Sexual Anatomy With Age

The tissues that make up the vulva are sensitive to hormones, estrogen in particular and therefore change in both anatomy and function with the surrounding endocrine environment. In infancy the vulva is devoid of hair. The young baby has plump labia majora and the vaginal epithelium is atrophied with a mean pH of 7.0. During childhood, the

skin of the vulva becomes thin and delicate with a reddish color. The growth of pubic hair is a marker for the onset of puberty, which usually happens between the ages of 10-12 years. At this point the vagina takes on the description previously provided. In old age all the tissues atrophy and the skin becomes noticeably drier. The subcutaneous fat lessens, and the labia minora shrink. The vaginal opening tends to contract and pubic hair becomes sparse. The rugae generally disappear as a result of coitus and childbirth and the vagina becomes a smooth walled canal (6,7,9).

3.0 Physiology of the Female Genital Tract

The main characteristic of the physiology of the female genital tract is Programmed change. These changes occur for one overriding purpose, procreation. This section aims to summarize both the long and short-term changes that occur in the female sexual organs as well as the reaction of the female gonads to sexual stimulation (7).

3.1 Short Term Changes

The most obvious of short-term changes in the female is menstruation. The menstrual cycle results from a complex chain of events modulated by hormonal and neuroendocrine events. The average menstrual cycle lasts about 28 days with a decrease in mean length as a woman ages. The menstrual cycle is divided into two phases. The first half is the follicular phase during which the mature oocyte is developed within an ovarian follicle. The second half is the luteal phase during which the active corpus luteum is derived from the ovulating follicle and produces estrogen. There are many organs responsible for orchestrating the events that result in menstruation. These include the hypothalamus, pituitary, ovaries, uterus and cervix (7,10).

3.1.1 The Hypothalamus and pituitary

The hypothalamus has a variety of functions including control of pituitary secretions. Releasing factors are

transported directly from the hypothalamus to the pituitary. One such releasing factor, Gonadotropin releasing hormone (GnRH) controls the release of pituitary gonadotropins such as Follicle stimulating hormone (FSH) and luetenizing hormone (LH). GnRH release frequency has been shown to increase from one pulse every 90-100 minutes during menses to one pulse every 50-60 minutes just prior to ovulation. The lower amount of GnRH causes a release of FSH from the pituitary whereas the increased frequency of GnRH pulses favors LH release. The continued GnRh secretion results eventually in the LH surge and ovulation. Following ovulation, GnRH release tends to slow down to one pulse every 4-6 hours. This slowing is largely caused by the presence estradiol and progesterone. With their eventual decline at the end of the luteal phase GnRH release frequency begins to increase again (7,10-12).

3.1.2 Ovary

The ovary serves as both a target for gonadotropins and as an endocrine gland, producing large amounts of gonadal steroids. Immature oocytes are stored within the ovaries in follicles. Prior to the onset of the menstrual cycle a follicle is recruited and begins development. Under the influence of FSH, the follicle develops further and is destined to ovulate. At the same time that the oocyte is maturing within the follicle, estradiol production has also

increased. The increasing estradiol levels down-regulate FSH release and up-regulate LH release. At the same time as the LH surge, the final maturation of the oocyte takes place. The follicular wall is digested away and ovulation occurs. The lifespan of the corpus luteum is approximately 14 days before degradation occurs. However, if pregnancy occurs, the increase in human chorionic gonadotropin (hCG) will allow the corpus luteum to function until a fetal placenta has developed (7,10).

3.1.3 Uterus and Cervix

The uterus and cervix provide the most visible evidence of reproductive cycling. The uterus is primarily a sac of muscle lined by endometrium. The endometrium undergoes characteristic biochemical and histological changes throughout the menstrual cycle. The first half of the cycle is characterized by proliferation of the endometrium. The endometrium is comprised chiefly of epithelial glands, stroma and blood vessels. These increase in thickness five to tenfold. Estrogen receptors, which are usually present in the uterus, also increase in number. This uterine proliferation continues until ovulation, when progesterone secretion inhibits further growth. Post ovulation, the endometrium begins to secrete glycogens. These are used to support the unattached blastocyst before implantation. If conception has occurred endometrial vessels continue to

grow. If pregnancy does not occur, estradiol and progesterone secretion falls resulting in ischemia to the upper two thirds of the endometrium. This results in a controlled shedding of the ischemic layer and menstruation.

The cervix also demonstrates certain characteristic changes throughout the menstrual cycle. The diameter of the cervical os changes under the influence of estradiol. It is normally constricted, however during times of peak estradiol levels the diameter increases. As well, the cervical mucus also changes in relation to estradiol levels. The mucus is generally thick and sticky. In the presence of estradiol the cervical mucus becomes clear and slippery facilitating the migration of sperm through the cervix in order to allow conception (7,10).

3.2 Long Term Changes

There are a variety of changes that occur over the lifetime of the female. These range from embryological development to pubertal changes and menopause.

3.2.1 Fetal and Neonatal Period

Germ cells migrate from the yolk sac to the gonadal ridge at 5 weeks gestation. Evidence of ovarian differentiation appears within 1-3 weeks. The germ cells undergo rapid mitosis and form 6-7 million oogonia. Female genital duct development is largely completed by the end of the first trimester with the upper two thirds of the vagina

differing in embryonic origin from the lower third. Exposure of the newborn to placental hormones ends at birth. This results in an immediate reflex rise in gonadotropins. Vaginal bleeding is common within the first week of life. The vaginal epithelium is well differentiated at birth. With the post-natal drop in steroid levels there is a mass exfoliation of the more mature intermediate cell layer (7,10,13).

3.2.2 Puberty

There is no precise signal for the onset of puberty although it normally occurs between 10 and 12 years of age. At this time, the hypothalamus begins to demonstrate increased secretory activity. In response to this, the ovarian follicles begin to mature. The increase in circulating estrogens results in the development of secondary sexual characteristics (breast development, pubic hair, etc…). In addition, the uterus grows and changes from a corpus to cervix ratio of 1:1 to 2:1. The first menstrual cycle usually occurs about two years after the onset of puberty, and the full maturation of the urogenital system may not be complete for several years after (7,10).

3.2.3 Adulthood

The most dramatic change that can occur in the adult life of a female is pregnancy. Although there is an

abundance of changes that the adult female undergoes during pregnancy they will not be discussed in this text. The second major changes that occur during adulthood in women is the menopause. The mean age of menopause in women in the U.S. is 51.4 years, the age at which they experience their last menstrual period. This change is attributable to the limited supply of ovarian follicles at birth. Evolutionarily, menopause probably developed as a mechanism to prevent pregnancy so late that the women would not be able to raise the child. Menopause is preceded by a period in which the ovaries begin to "fail". Menstrual cycles become less regular, estrogen levels decrease and a variety of physical and emotional changes occur i.e. hot flashes and depression. Other changes that occur as a result of dwindling estrogen levels are vaginal dryness and a gradual atrophy of the sexual organs. Sex drive is usually retained in post-menopausal women (5,7,10,14,15).

3.3 The Response to Sexual Arousal.

During sexual arousal genital vasocongestion occurs resulting in engorgement of the vulva, particularly the labia minora and clitoris. The vaginal canal is lubricated by secretions from uterine glands and via vascular transudate. Transudation is caused by an increase in pressure inside the vaginal capillary bed which results in transudation of plasma through the vaginal epithelium.

Additional moistening during intercourse comes from secretions from Bartholin's glands (7,12,16,17).

4.0 Neurophysiology of Female Sexual Arousal

In recent years, significant insight has been made into the neurophysiology that controls and is responsible for the characteristics of female sexual arousal. The majority of this knowledge comes from animal studies. Many aspects of the central and peripheral nervous system control female sexual arousal. These include sensory pathways, spinal reflexes, brainstem, hypothalamus and the forebrain (11,12).

4.1 Sensory Pathways

Sensory Stimuli are conveyed via the afferent branches of the pudendal, hypogastric and pelvic nerves. These nerves terminate in the dorsal horn as well as medial central gray of the lumbosacral spinal cord. A variety of experiments have been performed in order to elucidate the role played by these sensory pathways. In the cat, stimulation of the pudendal gives rise to spinal field potentials. Stimulation of genital afferents have also resulted in labeled neurons consistent with the distribution of pelvic sensory terminals. Neurotrophic viruses have been injected directly into the clitoris and these have been mapped to the spinal neurons originating in the central gray region of the lumbosacral spinal cord (6,11,12).

4.2 Spinal Reflexes

Spinal reflex is responsible for many of the observed characteristics of sexual function. The best studied spinal

reflex is the bulbocavernous reflex seen in both males and females. The low threshold pudendal sensory fibers activate the pudendal motor neurons and causes contraction of the striated perineal muscle. This reflex is often used as a neurological test of the pudendal nerve function. The exact function of the reflex is unknown however continual stimulation of the clitoris may lead to the development of the orgasmic platform.

Stimulation of the cavernous nerve in female rabbits results in an increase in clitoral pressure as well as clitoral and vaginal blood flow and increased vaginal length. There is also evidence that climax is also a spinal reflex. A significant number of spinal cord injured women are able to experience sexual climax as has been observed in anaesthezized and spinalized female rats (11,12).

4.3 Brainstem Regions

Spinal sexual reflexes are under both inhibitory and excitatory control from brainstem sites. One site that has been demonstrated to be important in males is the nucleus paragigantocellularis. It projects directly to pelvic efferent neurons, which innervate the penis and clitoris. Lesions of this nucleus suppress tonic inhibition of the climax like response. This response is described as vaginal and uterine contractions in females and perineal muscle contraction, erection and ejaculation in males. Given the

degree of similarity in this response between males and females it is likely that this forebrain region plays a similar role in females. This region of the forebrain also stains positively for serotonin. Serotonin, when applied to the spinal cord, inhibits spinal sexual reflexes also making it a likely cause of the high incidence of sexual dysfunction seen in individuals taking SSRI's. All of these findings point to the nucleus paragigantocellularis as being a main component in the control of sexual function (2,11,12).

4.4 Hypothalamus

As mentioned previously, the hypothalamus has a wide variety of functions. The hypothalamus is essential for reproduction and sexual behavior in addition to homeostatic and motivational functions. The medial preoptic area (MPOA) has long been known to play a major role in male sexual behavior. Lesions to the MPOA abolish male copulatory behavior. However, these lesions do not affect nocturnal erections or masterbation. Some researchers believe that the MPOA relates the ability to recognize a sexual partner. A similar condition may be true in females. Female rats suffering from MPOA lesions display a greater degree of lordosis, however they avoid sexual partners when given the choice.

The paraventricular nucleus (PVN) is another hypothalamic area known to affect sexual function. It was consistently labeled after pseudorabies virus injection into the clitoris of female rats. Neurons found in the PVN are activated during sexual arousal in the female. During sexual arousal and orgasm, oxytocin (a hormone which is elevated during sexual arousal) from the PVN is secreted from the pituitary (10-12).

5.0 Objectives

The global objective of the current experiments is to establish a model of sexual arousal in the female rat. I have attempted to do so by examining the physiology and the histology surrounding the lubrication-swelling response observed in the female rat. The same response observed in the rat when exposed to sexually exciting stimuli. This research has 3 specific objectives, which are reported in this dissertation.

Objective 1: The first objective is to establish whether electrical stimulation of the dorsal clitoral and pelvic plexus nerves would be able to increase the blood velocity in the clitoris and vagina of the female rat. (**Article #1**)

Objective 2: The second experimental objective is to examine whether infusion of specific neurotransmitters and their antagonists would modulate the response of clitoral blood velocity to electrical stimulation. (**Article #2**)

Objective 3: The third experimental objective is to examine the rat clitoris histologically for the presence of the aforementioned neurotransmitters endogenously within the female rat. (**Article #2**)

These objectives are described as they were in articles being submitted to International Journal of Impotence Research.

6.0 Article #1

Increases in clitoral and vaginal blood flow following clitoral and pelvic plexus nerve stimulations in the female rat

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Abstract

The objective of the present study is to establish a model in the rat for the study of female clitoral and vaginal vascular changes during sexual excitation. A laser Doppler was used to measure blood flow changes following clitoral and pelvic plexus nerve stimulations. Results show an increase in clitoral blood flow following clitoral nerve (df_1=12, df_2 =108, F= 21.4, p<0.001) and pelvic plexus nerve stimulations (n=3). A vaginal blood flow increase is also observed following pelvic plexus nerve stimulations (df_1=12, df_2 =108, F= 4.75, p<0.001). The female rat can therefore be used as a model for the study of the physiology, pharmacology and sexual dysfunction relating to blood flow in clitoral and vaginal tissue.

Keywords: rat, female, blood flow, laser Doppler, nerve stimulation

Introduction

Arousability in female sexuality is defined as the ability to attain and maintain a lubrication-swelling response during sexual activity [1]. Sexual vascular responses in females include erection of the clitoris and congestion of the lower third of the vagina with transudation of fluids on the vaginal surface [2]. Defects in neurovascular mechanisms that accompany arousability will lead to sexual dysfunction and consequently, to somatic complications and psychosocial burden in women.

The objective of the present study is to establish a model in the rat for the study of female clitoral and vaginal vascular changes during sexual excitation. A laser Doppler was used to measure blood flow changes following clitoral and pelvic plexus nerve stimulations. Data gathered from these experiments will establish baseline values for future physiological and pharmacological studies of sexual arousal as well as pathological changes occurring in female sexual dysfunctions.

Materials and Methods

Ten female Sprague-Dawley rats (Charles River, Canada) weighing between 250-300g were used for this study. Under general anesthesia with pentobarbitol (60mg/kg, MTC Parmaceuticals, Cambridge, Ontario) the dorsal clitoral nerves were isolated following a mid-line incision of the skin immediately cranial to the clitoris. A laser Doppler probe (diameter 0.5mm, Transonic Systems, Ithaca, NewYork) was placed on the surface of the clitoral glans to measure capillary blood flow. The reflected laser light is registered on a photodetector and is proportional to red blood cell velocity [3]. The probe was attached to a flowmeter (ALF-21, Transonic Systems, Ithaca, NewYork). The laser Doppler flowmeter was calibrated to an internal standard. A drop of mineral oil was placed at the tip of the probe for proper blood flow readings in units of ml/min/100g of tissue. Stimulations of the dorsal clitoral nerve as well as the ventral aspect of the neuro-vascular bundle (Figure 3,4) leading to the clitoris, were done while recording capillary blood flow changes with the laser doppler. Electrostimulation was performed with a stainless-steel bipolar hook electrode. Rectangular monophasic pulses were delivered by a signal generator (MacLab 8S stimulus isolator, ADinstruments, Australia). Stimulus parameters

were a current of 2 mA, frequency 20Hz, pulse width 0.2 ms and a duration of 5 s.

Following the recordings from clitoral tissue, the abdomen was incised and the symphysis pubis was cut to gain access to the full length of the vagina and to visualize the pelvic plexus nerves. For peritoneal vaginal wall recordings, a plastic tube with an external diameter of 0.5 mm, was placed within the vagina to distend it. Vaginal recordings were also taken while the laser doppler probe was placed directly in the vagina following the removal of the plastic tube.

Using a dissecting microscope (6x), the pelvic plexus nerves were isolated for electrostimulation. Nerves innervating the vagina are derived from the pelvic ganglion and are located in the mesometrium along with blood vessels [4,5]. Nerves were isolated and stimulated in a cranial to caudal fashion starting with the nerves innervating the external sphincter of the bladder. Upon stimulation, a contraction of the sphincter and the bladder could be clearly observed. Responses were recorded when nerve stimulations resulted in an increase in vaginal blood flow. Electrostimulation parameters were identical to those of the dorsal clitoral nerve stimulation.

Baseline recordings were taken every 5 s for 30 s prior to electrostimulation. Nerve stimulations lasted 5s and

blood flow was recorded every second. Following electrostimulation, blood flow was recorded every 5 s for a duration of 30 s. Statistical analysis was performed to compare baseline to electrostimulation blood flow values. For statistical analysis, a repeated measured design was used to calculate an F ratio.

Results

In ten female rats, clitoral blood flow changes following stimulation of the dorsal clitoral nerve are shown in figure 1. Results demonstrate an immediate increase in blood flow following stimulations (df_1=12, df_2 =108, F= 21.4, $p<0.001$). Average baseline and electrostimulation blood flow values are 9.44 ± 3.6 and 16.35 ± 5.8 ml/min/100g respectively. In three rats, clitoral blood flow increased from 8.97 ± 1.5 to 13.7 ± 1.94 ml/min/100g following pelvic plexus nerve stimulation. A decrease in blood flow from 8.4 ± 3.15 to 4.3 ± 1.35 ml/min/100g was observed following stimulation of the ventral aspect of the neuro-vascular bundle.

Vaginal blood flow was recorded from the laser probe placed on the peritoneal wall of the vagina. Results show an immediate increase in vaginal blood flow following pelvic plexus nerve stimulations (df_1=12, df_2 =108, F= 4.75, $p<0.001$) (Figure 1). Average baseline and electrostimulation values are 6.3 ± 4.7 and 25.8 ± 9.5 ml/min/100g respectively. In 3 rats where the laser Doppler probe was placed in the vagina to record blood flow changes following pelvic plexus nerve stimulation, increases in blood flow from 3.35 ± 0.22 to 8.35 ± 0.59 were recorded.

Discussion

Results show that clitoral and vaginal blood flow increase during the stimulation of the dorsal clitoral and pelvic plexus nerves respectively. Stimulation of the pelvic plexus nerves also leads to increase clitoral blood flow, however this was only demonstrated in three animals. To lend support to the methodology used and the results obtained in this study, decreases in clitoral blood flow were recorded following stimulation of the ventral aspect of neurovascular bundle leading to the clitoris. This can be explained either by a vasospasm in the clitoral artery or stimulation of sympathetic fibres.

The parasympathetic component of the autonomic nervous system is responsible for the clitoral swelling during sexual excitation as well as the lubrication, vasocongestion and lengthening of the vagina [2,6]. These physiological changes are associated with an increase in blood flow and would therefore be mediated by the parasympathetic system suggesting that pelvic nerve stimulations were performed on parasympathetic fibres.

Previous studies have demonstrated the importance of the male rat model in erectile function and dysfunction. In males, stimulation of the cavernous nerve or the dorsal penile nerve [7-12] have shown an arterial blood flow increases in the corpora cavernosa, and consequently an erection is

obtained. This increase in blood flow is triggered by smooth muscle relaxation. After sexual stimulation, women experience increases in vaginal lubrication and clitoral erection that are triggered by an increase in blood flow in pelvic organs. Our model has shown the same physiological response and will enable us to study sexual dysfunction.

Different neuromodulators have been identified that play a role in clitoral and penile erection as well as lubrication of the vagina during arousal. Nitric oxide has been thought of as the main neuromodulator implicated in the initiation and maintenance of penile erection [13]. Since there are many homologies between male and female sexual tissues [14], it is possible that nitric oxide is an important neurotransmitter in smooth muscle relaxation in the clitoris and vagina. In humans, nitric oxide synthase isoforms are present in clitoral [15] and vaginal [16] tissue and therefore nitric oxide donors may play a role in controlling blood flow in these tissues. Studies in laboratory animals [17-20] and farm animals [21] also show that nitric oxide may play a role in female genital organs such as neurogenic vasodilation and relaxation of smooth muscles. Other vasoactive peptides with relaxant properties, such as vasoactive intestinal polypeptide (VIP), have been shown to be present in the vaginal tissue of animals [22,23] and humans [24,25]. In humans, VIP increases blood flow in the vagina and provokes vaginal

lubrication [26-27]. No comparative study has been done in the same animal species to localise different vasoactive neuropeptides that are important in the regulation of vascular and smooth muscle control of the vagina and clitoris of female rats.

Increases in blood flow of clitoral and vaginal tissue during nerve stimulations has been shown in rabbits and dogs [28,29]. Using laser Doppler flowmetry in rabbits, clitoral and vaginal blood flow increases are 100% and 160% respectively following pelvic nerve stimulation. In female dogs, clitoral pressure measured directly with a needle placed in the corpus cavernosal tissue, show a 62% increase following cavernous nerve stimulation. Our results show that clitoral and vaginal blood flows increase by 37% and 309% respectively following stimulations of the clitoral and pelvic plexus nerve stimulations. The female rat is therefore a good model for the study of human sexual dysfunction relating to different pathologies affecting the pelvic vasculature.

Most sexual problems that occur in menopausal women are due to inadequate lubrication caused by estrogen deprivation[30]. Estrogens have been associated with an increase in the vascular bed of sexual organs. A better understanding of the neurovascular control and physiology of sexual organs in aging female rats could lead to new

therapies to problems such as dyspaneuria and vaginal dryness.

Sexual problems have also been documented in diabetic women. A review on diabetes and sexuality has been published by Enzlin *et al* [31]. Diabetes is a well-known cause of male impotence and therefore it is not surprising that similar sexual dysfunction would be present in diabetic females. Impaired sexual function in diabetes mellitus causes problems with vaginal lubrication and has no influence on orgasm. Diabetic women have problems with sexual arousal and this being twice the prevalence observed in the general population.

Very few studies have been done to understand the physiological changes in clitoral and vaginal tissue associated with female sexual function. Another female rat model for the study of sexual function has been established by McKenna *et al.* [32]. In this model vaginal and uterine contractions and rhythmic contractions of the perineal muscles were recorded following urethral stimulation. The use of the urethrogenital reflex gives a valuable model for the study of neural mechanisms of sexual function. Since these studies mainly focus on the neural control of smooth and striated muscle contractions it would be of interest to measure simultaneously vascular and muscular changes during

the urethrogenital reflex in normal and pathological animals.

Our results show that blood flow changes can be measured in the female rat during nerve stimulation. The rat can therefore be used as a model for the study of physiology, pharmacology and sexual dysfunction relating to blood flow of clitoral and vaginal tissue. Compared to other animal models mentioned in this paper, the female rat seems a more interesting model to study female arousability since a greater number of systemic and genetic anomalies are available in this species and because of the lower costs for purchase and maintenance.

Figures

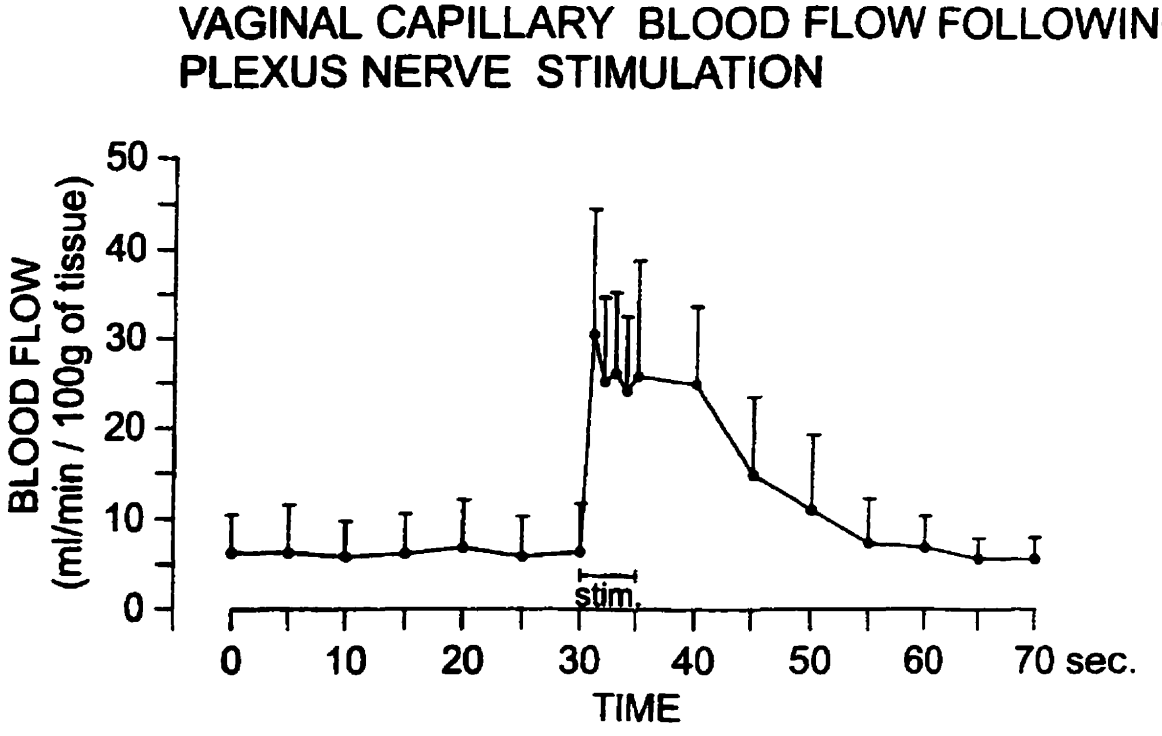

Figure 1. Vaginal blood flow changes recorded before and after electrical stimulations of the pelvic plexus nerves. The laser Doppler probe placed on the peritoneal wall of the vagina. (Stim.= duration of electrical stimulation). Blood flow during electrical stimulation are statistically different from baseline values($p<0.001$).

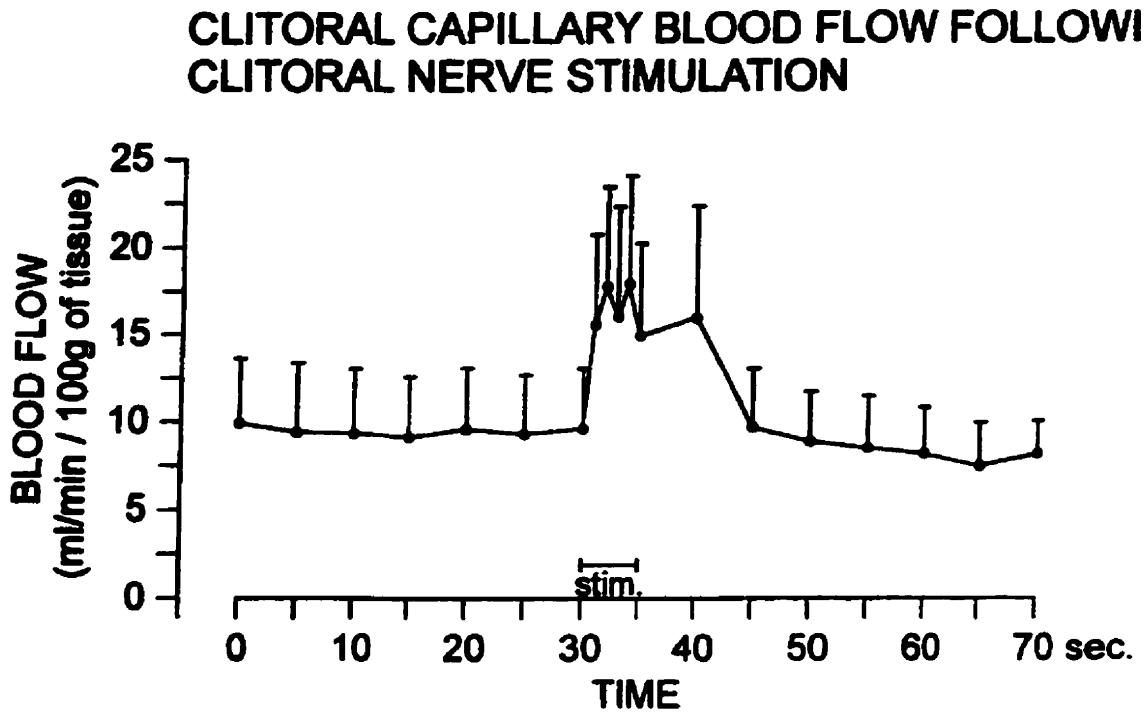

Figure 2. Clitoral blood flow changes recorded before and after electrical stimulations of the dorsal clitoral nerves. The laser Doppler probe placed directly on the clitoris (Stim. = duration of electrical stimulation). Blood flow during electrical stimulation are statistically different from baseline values
($p<0.001$).

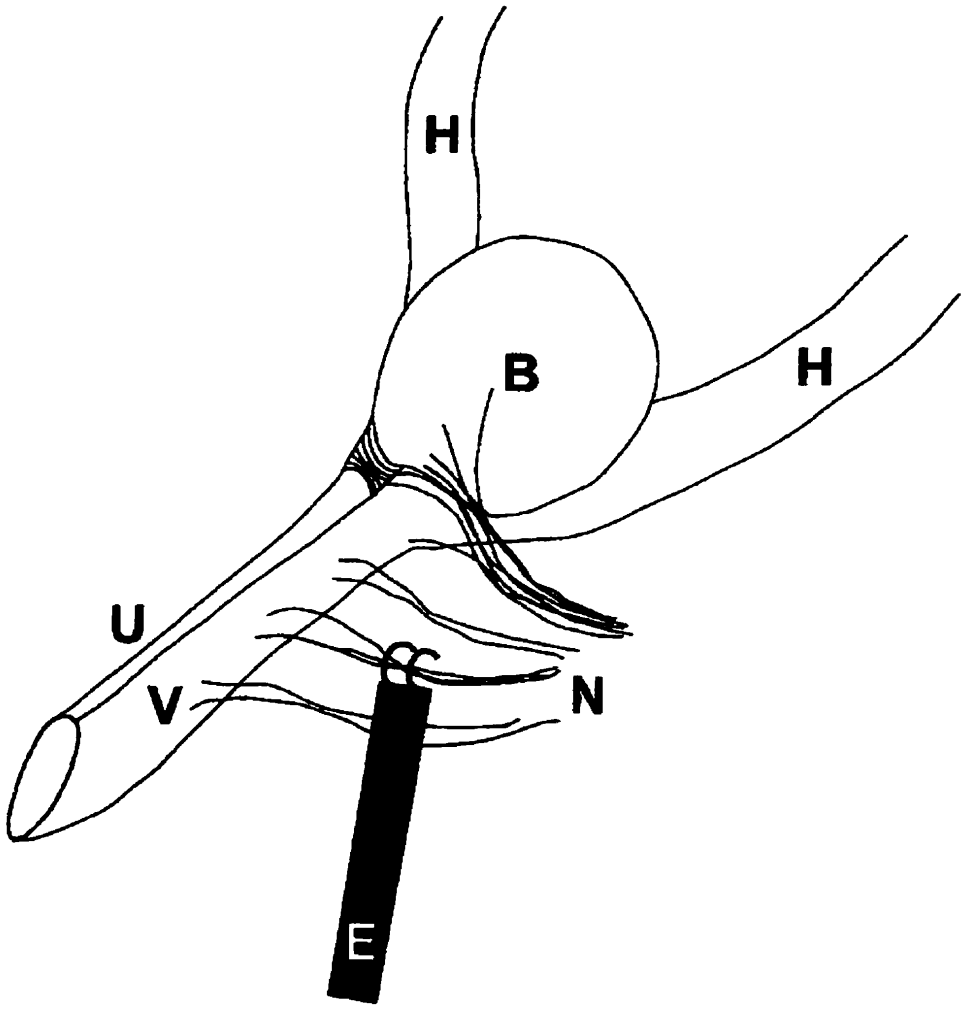

Figure 3. Schematic drawing showing pelvic plexus nerve innervation to the vagina and bladder of a female rat. The stimulating electrode (**E**) is placed on post-ganglionic fibers dissected from the mesometrium. **V** vagina, **N** nerves, **U** ureter, **B** bladder,**H** uterine-horn.

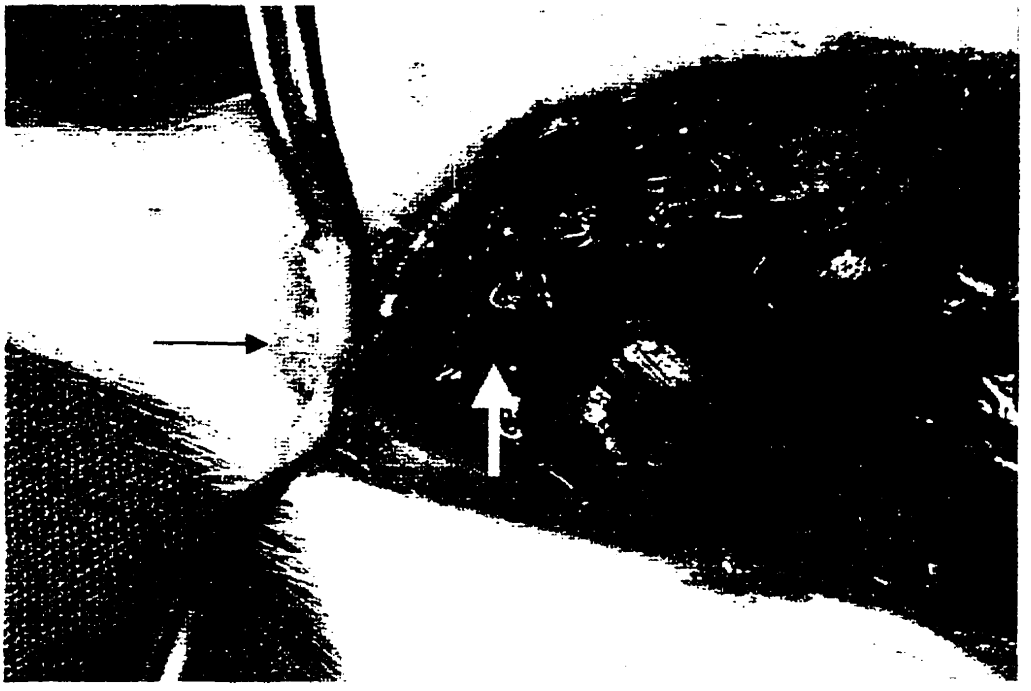

Figure 4. Photo of the clitoris (small arrow) and neurovascular bundle (large arrow) of a female rat.

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7.0 Article #2

Female Sexual Dysfunction: A Vasculogenic Model In The Female Rat.

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Introduction

The recent advances in pharmacological treatment of erectile dysfunction have increased the interest in the study of female sexual dysfunction (FSD). Females do indeed suffer from a wide range of pathologies that can affect sexuality, which are both similar and unique to those pathologies suffered by males. These include, but are not limited to; those brought on by neurodegenerative disorders, vascular disorders and advancing age (1,2). Studies comparing sexual dysfunction amongst "normal" couples (3) revealed that 10-52% of males suffer from erectile dysfunction whereas 25-63% of females suffer from some type of sexual dysfunction. Sexual dysfunction in women usually presents as Female Sexual Arousal Disorder (FSAD), Female Orgasmic Disorder (FOD) or Vaginismus. Despite being common, only recently have physicians and researchers begun to properly acknowledge FSD.

Sexual arousal in the female is characterized physiologically by an increase in blood flow to the clitoris and vagina resulting in vaginal lubrication and clitoral engorgement, known as the lubrication-swelling response (4,5). It is believed that this increase in blood flow is a result of local neuromodulation on the capillary beds by neurotransmitters such as nitric oxide (NO), vasoactive

intestinal peptide (VIP) and calcitonin gene related peptide (CGRP) (6-8). A number of models have been developed to study FSD. Most of these have used animals such as dogs or rabbits and have involved both direct and indirect examination of blood flow and neural activity in these organs (4,5,9). We have previously reported our successful attempt at blood velocity modulation and recording in the female rat (10). Our vasculogenic model in the female rat is based upon electrical stimulation and laser Doppler flowmetry. We have created a model that allows us to measure the vascular response to electrical stimulation of the sex organs in the female rat. The use of the female rat is appealing given that the many studies in male sexual function has been performed in the rat and are well documented (4,11,12). We benefit by being able to compare a model of sexual function between the male and female of the same species. In addition, the wide range of pathologies and genetic manipulations already established in the rat make it an ideal candidate to study the effects of these conditions on FSD.

The global aim of this study is to examine the effects of various neurotransmitters on the sexual arousal response in female rats. Our first specific aim is to measure the effects on blood velocity in the clitoris in response to electrical stimulation combined with the infusion of NO

donor, VIP and CGRP and their respective antagonists. These neurotransmitters have been implicated in the relaxation of the smooth muscle in the corpora cavernosa of the penis resulting in erection in males (2,13). They have also been localized to within the neurons that innervate the human clitoris and vagina and are believed to play a role in the lubrication-swelling response observed during sexual arousal (14-17). The second specific aim of the study was to examine the rat clitoris histologically for the presence of both endothelial Nitric Oxide Synthase (eNOS) and neural Nitric Oxide Synthase (nNOS), the enzymes responsible for synthesizing NO, as well as VIP and CGRP.

Materials and Methods

Animals

Female Sprague-Dawley rats (250-300g, Charles River, Quebec) were used for the experiment. They were housed in a standard 12h/12h light/dark cycle animal housing facility with access to food and water *ad libitum*. Upon receipt of the animals they were allowed a 5-day acclimatization period before any experiments were performed. The rats were subdivided into 5 groups; SNP (n=5), VIP (n=5), CGRP (n=6) and Control (n=5). The institutional animal care committee prior to experimentation approved the protocol.

Surgery and Data Collection

Surgery and Data collections were performed in the same manner previously reported (10). Rats were anesthetized with pentobarbitol, (50 mg/kg, MTC pharmaceuticals Cambridge, Ontario) prior to surgery. Upon anesthesia, a midline incision immediately superior to the thyroid gland was made. Using a dissecting microscope (Leica instruments) the carotid artery was isolated and cannulated with PE-50 tubing attached via a pressure transducer to a MacLab 8S biomonitoring system (AD instruments, Australia) for *i.v.* drug infusion. Following cannulation of the carotid artery, a midline incision of the skin immediately cranial to the clitoris was made and the dorsal clitoral nerves were isolated. A laser Doppler flow probe (diameter 0.5mm,

Transonic System, Ithaca NY) was placed on the surface of the glans clitoris to measure any change in capillary blood velocity. The probe was attached to a flowmeter (ALF-21, Transonic Systems, Ithaca NY) and calibrated to an internal standard. A drop of mineral oil was placed at the tip of the probe for proper blood velocity recording in units of ml/min/100g tissue. The flowmeter was coupled to the MacLab 8S, and the data recorded. Data was recorded for the ten seconds prior to stimulation, during stimulation and the ten seconds post-stimulation. A signal generator (MacLab 8S stimulus isolator, Adinstruments, Australia) delivered rectangular monophasic pulses. Stimulus parameters were a current of 2mA, frequency 20Hz, pulse width 0.2ms with duration of 10s. During the experiment the capillary blood velocity was recorded four times. The first recording was made following electrical stimulation without neurotransmitter (NT) infusion. The second capillary blood velocity recording was after infusion of the NT agonist and following electrical stimulation. The third recording was made following infusion of the NT antagonist and electrical stimulation. Time control animals underwent the same procedure but were infused with a normal saline solution (0.9% NaCl) instead of NT agonist or antagonist. Blood velocity was allowed to return to resting in-between recordings.

The estrous phase of the rats were determined by examining a smear of fluid taken from within the vagina of the rat. The smear was stained with Gemsa and examined underneath a light microscope.

Drug and Dosage of agonists and antagonists of NO, VIP and CGRP

All drugs were purchased by Sigma Chemical (St.Louis, MO) and administered intravenously in a bolus dose over the course of 1-2 minutes. **SNP**: Sodium Nitroprusside (SNP) was used as a NO donor (8µg/kg dissolved in normal saline). N^G-nitro-L-arginine methyl ester (L-NAME) was used as NOS inhibitor (20mg/kg dissolved in dist. H_2O) (18-21). **VIP**: Both VIP and VIP antagonist VIP-Cl-Phe^6-Leu^{17} were dissolved in de-oxygenated H_2O (VIP: 1µg/kg, VIP-Cl-Phe^6-Leu^{17}: 60µg/kg) (22,23). **CGRP**: Human CGRP (100pmol/kg) and the specific CGRP antagonist, $CGRP_{8-37}$ (50nmol/kg) were dissolved in dist. H_2O(23-25).

Tissue Collection

Rats were perfused intracardially with 60ml of normal saline (0.9%) followed by infusion of 60ml of 4% paraformaldehyde. Perfusion was performed by incision into the thoracic cavity along the midline, followed by introduction of the perfusate into the left ventricle through an 18G hollow bore needle after which the right atrium was punctured. After perfusion, the clitoris was

dissected from the animal. The removed organ was placed in 4% paraformaldehyde at 4°C overnight after which they were removed from the solution, frozen in liquid nitrogen and stored at -70°C.

NADPH Immunohistochemistry

Frozen tissue was cut on a cryostat into 16μm sections along the transverse axis (Figure 1). Sections were placed in phosphate buffer saline (PBS) (pH 8.0) for 15 min. and left to dry for 5 min. at room temperature. Sections were then placed in a 0.1 M PBS (pH 8.0) bath containing NADPH (0.5mg/ml) nitroblue tetrazolium (0.2mg/ml) and 1.0% Triton for 5 hours. Sections were then washed in PBS, dehydrated in graded ethanols, cleared in xylenes and coverslipped.

VIP and CGRP Immunohistochemistry

Frozen tissue was cut on a cryostat into 16μm sections along the transverse axis. Sections were placed in PBS and 0.3% Triton for 15 min. at room temperature followed by a 30 min. bath in 0.3% normal goat serum. Without rinsing, sections were placed in VIP or CGRP antibody solutions (1:100) overnight at room temperature. Sections were then washed in three consecutive 10 min. baths of PBS and placed in Fluorescein Isothyocyanate (FITC) conjugated goat anti-rabbit IgG (1:100) for 10 min. at room temperature. Sections were then washed in PBS, dehydrated in graded ethanols, cleared in xylenes and coverslipped.

Results

NO donor

Clitoral blood velocity was modulated by the infusion of SNP (Graph 1). The mean clitoral blood velocity before neurostimulation (**at rest**) was 13.36 ml/min/100g. Clitoral nerve stimulation prior to the infusion of any neurotransmitters (**baseline**) resulted in a mean increase in clitoral blood velocity of 42.9% ± 12.6% from resting, $p<0.05$. Stimulation after the infusion of SNP resulted in a mean blood velocity increase of 81.7% ± 21.3% from resting. This increase was significantly higher than that observed during the baseline stimulation, $p<0.05$. Stimulation after infusion of L-NAME resulted in a 16% ± 9.3% increase in blood velocity from rest. This increase was significantly lower than that observed after stimulation with SNP $p<0.05$. There was no statistically significant difference in clitoral blood velocity response between the stimulation with L-NAME and the baseline stimulation.

VIP

Clitoral blood velocity was effectively modulated by VIP and its specific antagonist (Graph 2). Mean clitoral blood velocity prior to electrical stimulation (**at rest**) in the VIP group was 20.58 ml/min/100g. Stimulation prior to the infusion of any neurotransmitter (**baseline**) resulted in

a mean increase in clitoral blood velocity of 42% ± 9.4%. This was significantly higher than the resting velocity, $p<0.05$. Electrical stimulation after the infusion of VIP resulted in a velocity increase of 71.6% ± 12.1% from rest. This increase was significantly higher than the baseline stimulation, $p<0.05$. Electrical stimulation in the presence of the VIP antagonist resulted in mean increase 41.46% ± 19.2% from resting. This increase was significantly less than that observed during stimulation in the presence of VIP. There was no statistical difference between the baseline increase and the VIP-antagonist increase.

CGRP

Infusion of CGRP in the female rat did not appear to have any significant effect on clitoral blood velocity (Graph 3). The mean resting clitoral blood velocity (**at rest**) was 20.68 ml/min/100g. Stimulation prior to neurotransmitter infusion (**baseline**) resulted in an increase of 34.6% ± 7.1% in mean clitoral blood velocity. Stimulation after infusion of CGRP resulted in a mean increase of 54.6% ± 10.9%. There was a lack of a statistically significant difference in between the increase observed without and the increase observed with CGRP. Infusion of the CGRP antagonist followed by stimulation resulted in a mean blood velocity increase of 21.9% ± 7.6.

This increase was significantly lower than that observed in the CGRP infused group ($p<0.05$).

Controls

The intensity of the vascular reaction did not change over time following saline infusion. (Graph 4). The mean velocity prior to any infusion in the control group was 17.9 ml/min/100g. Stimulation prior to infusion of saline resulted in a mean velocity increase of 29.7%. Stimulation after the first saline infusion resulted in a mean velocity increase of 26.4%. Stimulation after the second saline infusion resulted in a mean increase in velocity of 32.4%. There was no significant difference observed between any of the stimulations.

Histological sections of the clitoris stained positive for eNOS (Figure 2), nNOS (Figure 3), VIP (Figure 4) and CGRP (Figure 5). Staining was most intense for the NOS stained sections and decreased in intensity for VIP. CGRP stained sections had the lowest intensity of staining.

Discussion

We have previously reported the successful modulation of clitoral blood velocity in response to stimulation of the clitoral nerves (10). Our model has proven to be a reliable measurement of the sexual arousal response in the female rat. Previous studies have outlined the importance of NO, VIP and CGRP on the erectile function in the male (2,11) as well as clitoral perfusion and cavernosal pressure in female dogs, rabbits and humans (5,9,14). The present study is a model in the female rat of the vascular and neurochemical control of sexual arousal.

Many researchers believe that NO is the "major player" in penile erection. NO is responsible for the neurogenic vasodilation necessary for engorgement of the corpora cavernosa that results in erection (2,26). Previous studies indicate that NO may play a role in the vasodilation within human female genital organs (27). All three isoforms of NOS (endothelial, neural and inducible) have been localized to the clitoris of human females within neurons that innervate the blood vessels and smooth muscle cells (7). In the present study we demonstrate that SNP, a potent NO donor, is able to augment the vasculogenic response to electrical stimulation in the clitoris of the female rat. The increase in blood velocity caused by electrical stimulation in the presence of SNP was significantly greater than the increase

generated by electrical stimulation alone. These data demonstrate the importance of NO in the vasodilation that occurs in the clitoris. When we increased the availability of NO, we observed an increase in blood velocity. When we stimulated the clitoral nerve in the presence of L-NAME the response was considerably lower. We observed only a very small change in blood velocity indicating that the antagonistic effects of L-NAME were blocking the vascular dilatory response induced by stimulation.

L-NAME is a selective antagonist of NOS and therefore not a direct antagonist to the exogenous NO supplied by SNP. Rather, L-NAME blocks the effect of endogenous NO. Infusion of L-NAME did block the vascular response to stimulation, but not completely. This may be attributable to the notion that L-NAME was not administered in a high enough dose. However, it is more likely that there are other accessory neurotransmitters involved, which are responsible for vasodilation, but not as potent as NO. This is also supported by the fact that NOS staining was more intense than VIP and CGRP. These results indicate that NO is probably important, but not the only neurotransmitter that affects the clitoral vasodilation observed during sexual arousal in the female. Further experimentation should focus on a dosage control trial of SNP and L-NAME. Administration of SNP and L-NAME concurrently would also be of interest.

VIP has been shown to increase blood flow in the corpora cavernosa of the penis (2). It is also involved in relaxation of vascular smooth muscle, vasodilation and secretion in the female genital tract (14,15). VIP is present in the presynaptic nerve terminals that innervate the clitoris and vagina of human females and has been shown to have a decreased potency in post-menopausal women (28). In our results, we observed that infusion of VIP combined with electrical stimulation increased clitoral blood velocity significantly higher than the baseline stimulation. While the observed blood velocity increase was not as high as that observed in the presence of SNP, VIP also appears to be very potent in the regulation of clitoral blood velocity. We have also observed that systemic infusion of VIP-Cl-Phe^{6}-Leu^{17}, a selective VIP antagonist, blocked the effects of VIP agonist infusion. When we stimulated in the presence of VIP-Cl-Phe^{6}-Leu^{17} the augmentation in the clitoral blood velocity caused by VIP was effectively blocked. This resulted in a response similar to that observed without infusion of any neurotransmitters. The VIP antagonist was able to block the agonistic effect of VIP on the blood velocity response to stimulation. This observation also supports NO as a principal mediator as velocity was able to increase to the same levels as baseline stimulation even though VIP action was being antagonized.

CGRP is a non-adrenergic non-cholinergic (NANC) general autonomic vasodilator that has been localized within nerves of the human female genitourinary tract and in the present experiment, to within the rat clitoris. Intracavernosal CGRP injections can enhance the rigidity of an already erect penis (11). CGRP on its own has not yet been found to be sufficient an agent to initiate penile erection. Our results demonstrate that the presence of CGRP did not affect the outcome of electrical stimulation of the dorsal clitoral nerves compared to the outcome achieved after baseline stimulation. Infusion of $CGRP_{8-37}$, a CGRP specific antagonist, caused a significant decrease in the clitoral blood velocity response compared to the CGRP infused group. When stimulated after infusion of $CGRP_{8-37}$, the clitoral blood velocity increase was only 21.9%. This was significantly lower than the increase observed after CGRP infusion, which was 54.6%. While CGRP itself is unable to significantly modulate the response to electrical stimulation, the CGRP antagonist demonstrates that CGRP may play a minor role in modulating the blood velocity. This effect is made apparent upon antagonizing CGRP and stimulating electrically. Doing so, we see that antagonizing the endogenous CGRP causes a decreased response to stimulation. As in males, it appears that the role of CGRP in the response to sexual arousal is as an accessory

rather than central. This is further supported by the fact that CGRP stained considerably less than NOS and VIP.

The control data acquired during this experiment demonstrate no significant differences in the vascular response between each timed stimulation. This indicates that the observed results were due likely due to the infused drugs rather than the course of time or anaesthesia, etc... These data were important in establishing the validity of our observations. In addition, the animals in this group were in different phases of the estrous cycle. Of the 5 control animals, 2 were in estrous, 1 in proestrous, 1 in metestrous and 1 in diestrous. There were no significant differences noted in-between the response of each animal. Although this may indicate that the estrous state of the rat is not a factor that affects the hemodynamic arousal reaction, this data is preliminary and must be investigated more thoroughly.

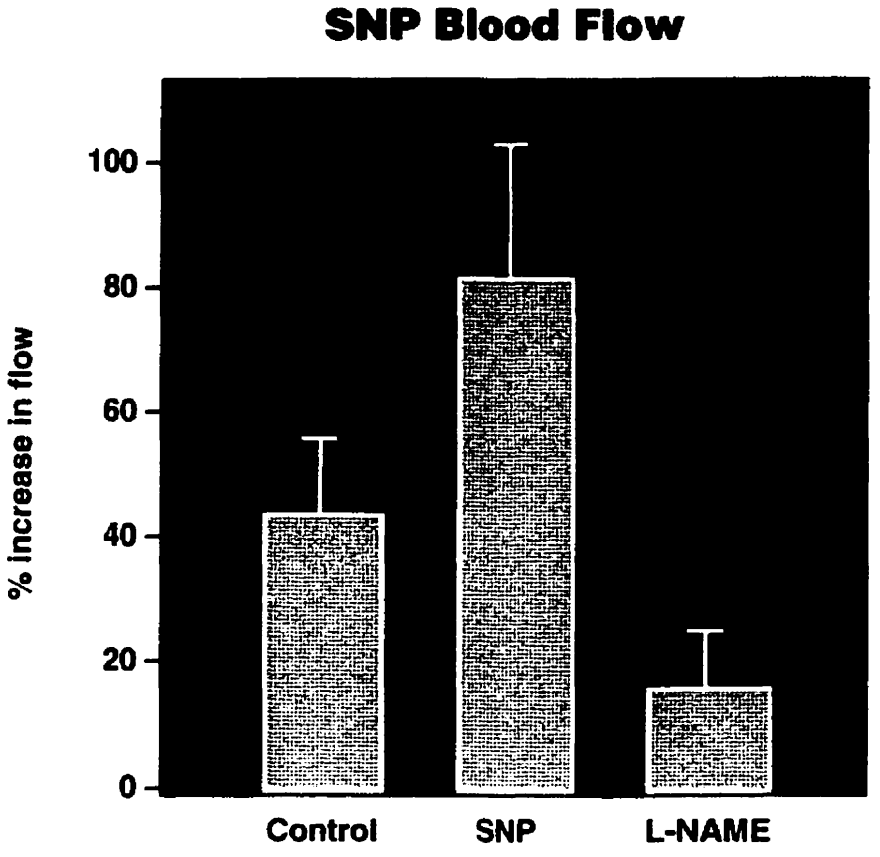
NOS, VIP and CGRP were all found to be localized within the clitoris of the female rat. These findings support these neurotransmitters as being responsible for vascular responses of sexual arousal. Our infusion data suggests however that NO may be the most important.

The limitations of the experiment are important to recognize. Unlike similar male experiments that allowed for direct intracavernous infusion of the neurotransmitters, the

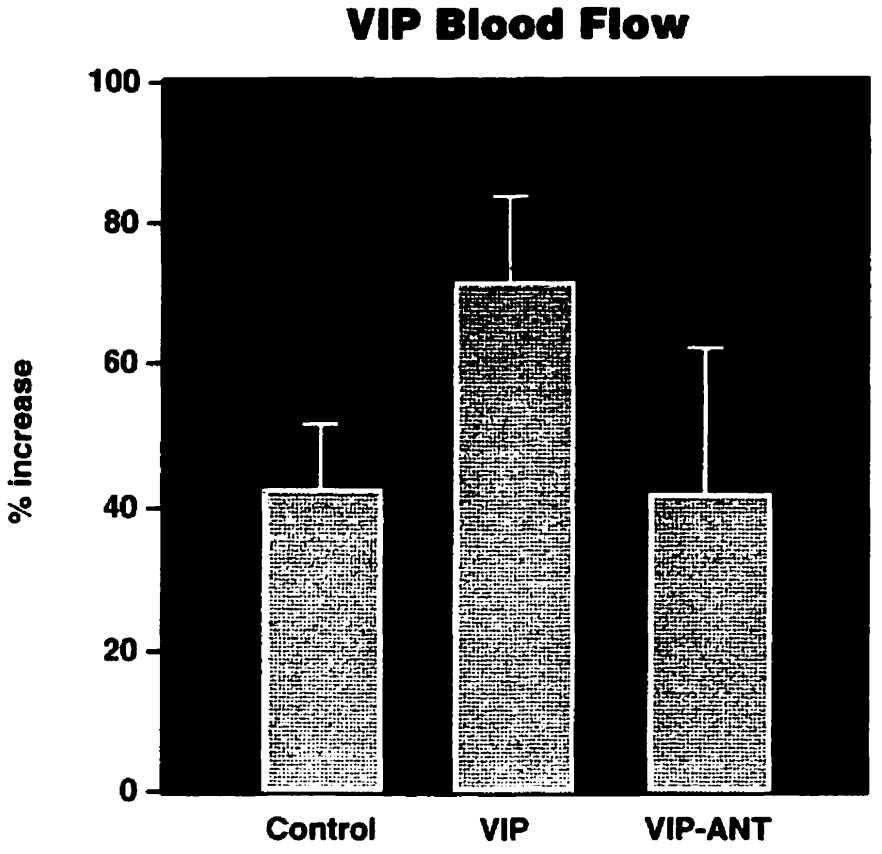
present experiment relied on systemic infusion. This results in a dilution of the neurotransmitter as it circulates systemically before it reaches the clitoris and a decrease effect. While almost impossible to inject directly into the corpora of the rat clitoris due to its small size, further experiments should most likely infuse more locally, i.e. internal iliac artery.

The present data must be examined further. It would be interesting to infuse an NO blocker followed by VIP and stimulate electrically or perform the reverse, infuse a VIP blocker and infuse with an NO donor followed by an electrical stimulation. This would allows us to examine the inter-relationship between NO, VIP and clitoral engorgement. In addition, dose response studies must be done for SNP, VIP and their antagonists.

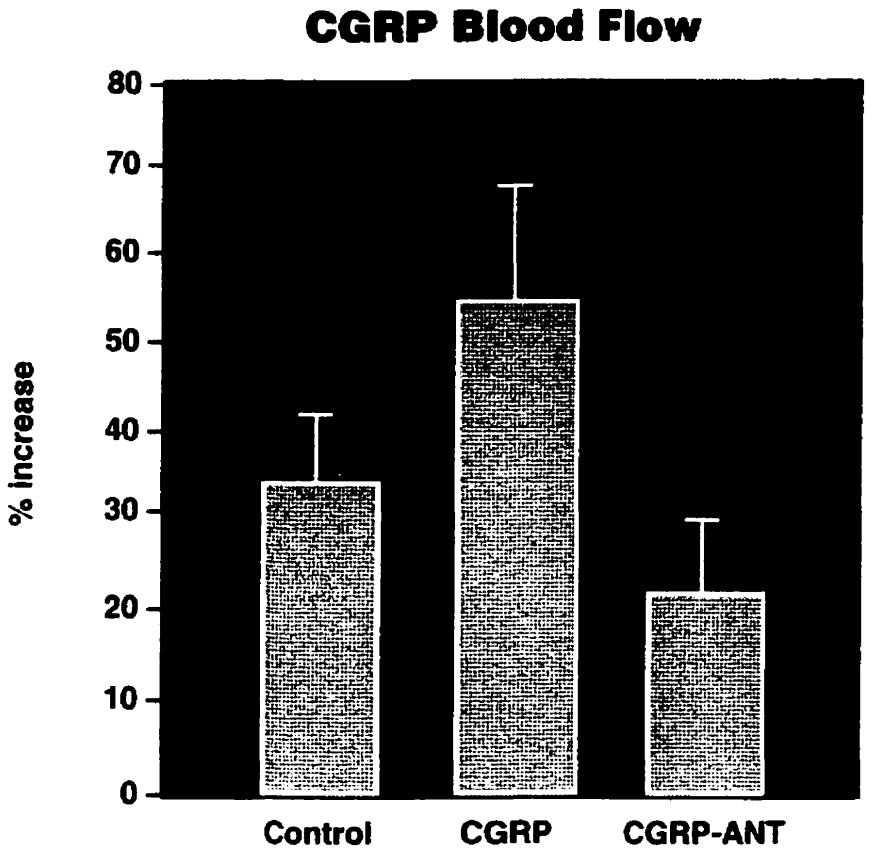
Graphs and Figures



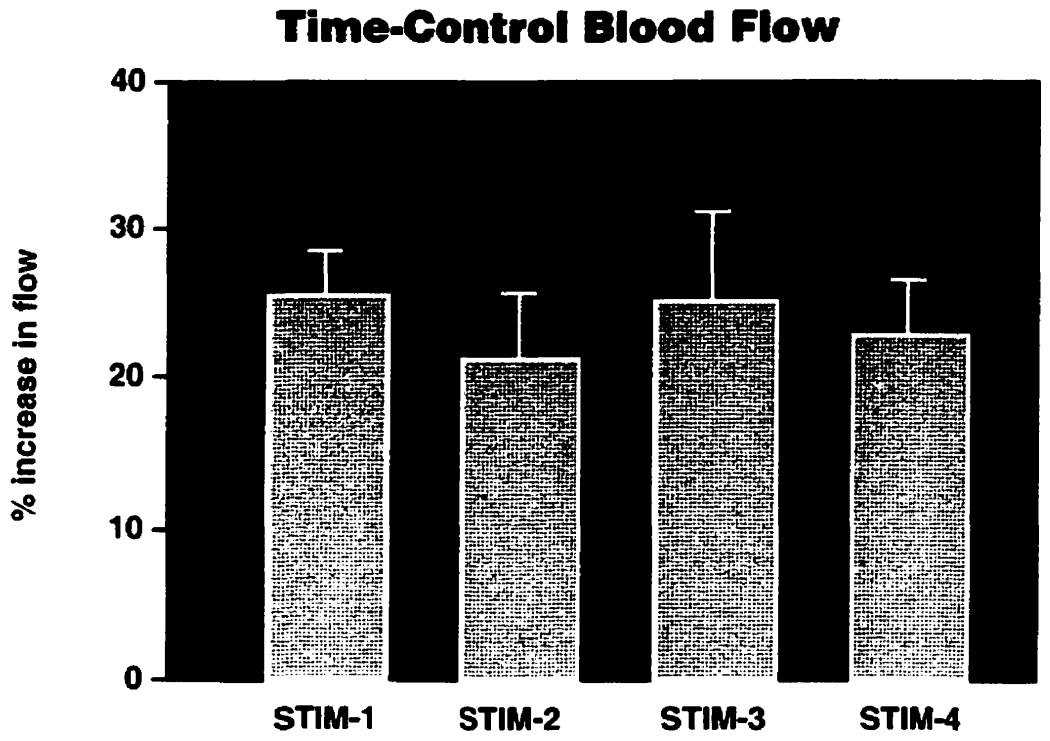
Graph 1. Percentage increase in blood velocity from rest during baseline stimulation (Control), stimulation with SNP and stimulation with L-NAME. Note that the SNP increase is significantly higher than control and the L-NAME increase is significantly lower than SNP, $p<0.05$.



Graph 2. Percent increase in blood velocity from rest during baseline stimulation (Control), stimulation with VIP and stimulation with the VIP-antagonist (VIP-ANT). Note that the VIP increase is significantly higher than control and the VIP-ANT increase is significantly lower than VIP, $p<0.05$.



Graph 3. Percent increase in blood velocity from rest during baseline stimulation (Control), stimulation with CGRP and stimulation with the CGRP-antagonist (CGRP-ANT). Note that the CGRP increase is not significantly higher than control($p>0.05$). However, the CGRP-ANT increase is significantly lower than the CGRP, $p<0.05$.



Graph 4. Percent increase in blood velocity during the four control stimulations. STIM-1 is baseline stimulation. STIM-2, 3 and 4 are after time delays and infusion of saline. There was no significant difference between any of the increases, $p>0.05$.

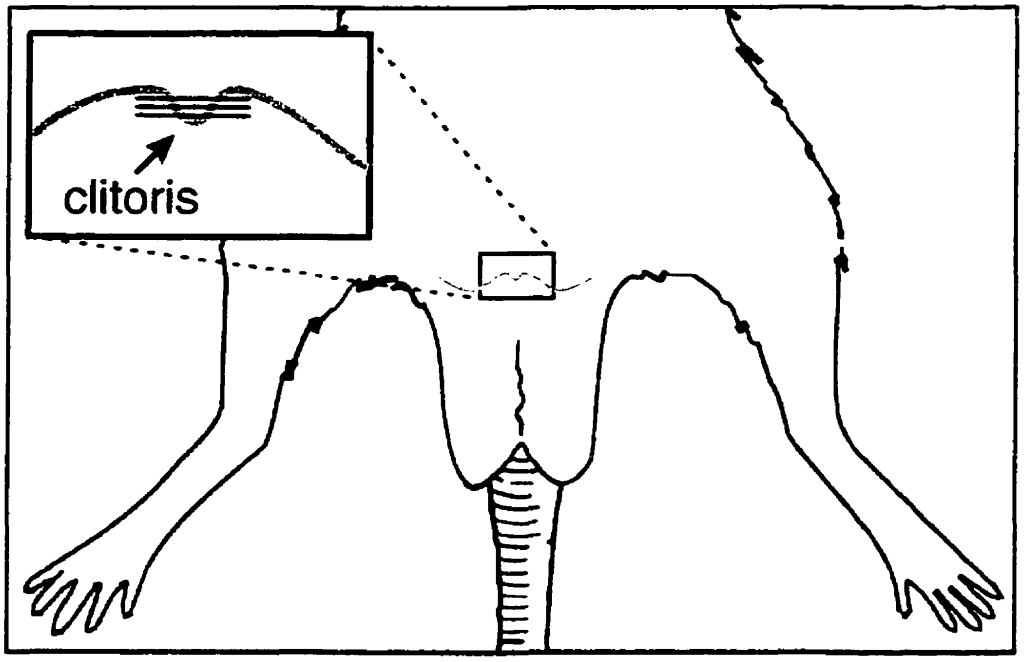


Figure 1. Graphical depiction of the clitoris of the rat as seen during surgery. Inset demonstrates the manner in which the clitoris was sectioned for histochemistry.

Figure 2. Fluorescence labeled eNOS positive stained endothelial cells lining blood vessels in a cross-section of the clitoris viewed at 100X.

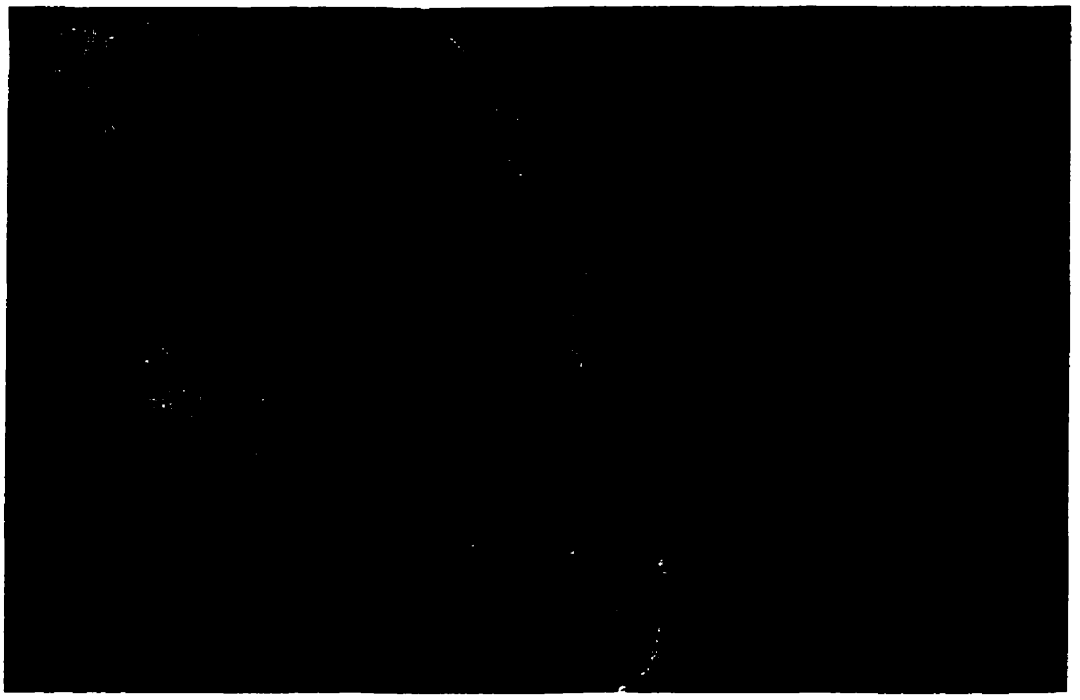


Figure 3. Fluorescence labeled nNOS positive stained fibers in a cross section of the clitoris viewed at 100X.

Figure 4. Fluorescence labeled VIP positive stained fibers in a cross section of the clitoris viewed at 250X.

Figure 5. Fluorescence labeled CGRP positive stained fibers in a cross section of the clitoris viewed at 250X.

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8.0 Etiology and Treatment of FSD.

8.1 Etiology of Female Sexual Dysfunction.

Female Sexual Dysfunction is a widely prevalent disorder with a large variety of causative factors. In addition to the many factors that can cause FSD, there are also many ways that FSD can present itself within a patient. Clinically, there are 5 main categories of female sexual dysfunction: 1-Vasculogenic, 2-Neurogenic, 3-Secondary to hormonal/endocrine problems, 4-Psychogenic and 5-congenital organ dysfunction.

8.1.1 Vasculogenic Female Sexual Dysfunction.

Clitoral and vaginal insufficiency syndrome are related directly to a reduced genital blood flow. The cause of the diminished flow can usually be attributed to the same catalysts that cause vascular problems elsewhere in the body. They are elevated blood pressure and cholesterol, smoking and heart disease. A decrease in pelvic blood flow may result in vaginal and clitoral smooth muscle fibrosis and dyspareunia. In addition, external factors such as bike riding or surgical disruption may cause damage to the iliohypogastric/pudendal arterial bed resulting in diminished flow to the sexual organs and resultant sexual dysfunction (2,3,5,18).

8.1.2 Neurogenic Female Sexual Dysfunction.

Any insult to the central or peripheral nervous system may result in neurogenic etiologies that can cause sexual dysfunction. Spinal cord injury or neuropathic disease, such as diabetes or Parkinson's, may result in an inability to achieve psychogenic lubrication. Women with spinal cord injuries tend to have greater difficulty achieving orgasm than normal women (2,3,5,18).

8.1.3 Female Sexual Dysfunction Secondary to Hormonal/Endocrine Problems.

Dysfunction of the hypothalamic/pituitary axis, menopause and premature ovarian failure are the most common causes of hormonally based FSD. The most common complaints in this category are decreased libido, vaginal dryness and pain and decreased arousal (2,3,5,18).

8.1.4 Psychogenic Female Sexual Dysfunction.

Whether there is organic disease present or not is not always the determining factor in FSD. Emotional and relational issues significantly affect sexual arousal in women. Personal issues such as body image, self esteem and relationship as well as psychological disorders such as depression can affect the sexual response. Psychotropic drugs can also cause FSD. Women taking SSRI's often

complain of decreased desire, arousal and difficulty achieving orgasm (2,3,5,18).

8.1.5 Congenital Organ Dysfunction.

There are a variety of gross problems that can arise to the sexual organs themselves resulting in FSD. Congenital malformations or lack of proper development of the vulva often result in the inability for physical arousal or coitus to occur. Cultural ceremonies such as female circumcision often result in mutilation of the genitals. Physical trauma such as this usually causes irreversible damage to the clitoris and vagina as well as severe psychological trauma.

8.2 Treatment Options for FSD.

Treatment for FSD is evolving at a rapid pace as clinicians and basic researchers are beginning to address this issue with greater concern. Most current treatment regimens revolve around hormone replacement therapy and/or standard psychotherapy. However, there are currently studies in progress assessing the efficacy of various vasoactive substances on the female sexual response. These substances include, but are not limited to, Methyltestosteron, Sildenafil, L-Arginine, Prostaglandin-E1 and Phentolamine (2,3).

The ideal approach to the treatment of FSD is a collaborative effort between basic scientists, clinicians

and therapists. Treatment of FSD is not a simple feat. It is important to note that there are many variables that must be analyzed before beginning medical therapies or determining treatment efficacy (19).

9.0 Importance Of The Rat Model Of Female Sexual Arousal

9.1 Why an Animal Model of Female Sexual Arousal?

Female suffer from sexual dysfunction. Statistics demonstrate that there is a higher prevalence of sexual dysfunction among women than among men (1,18). The prevalence of female sexual dysfunction has been shown to increase with risk factors such as age, vascular disease and neuropathic disorders (2,3). Despite the widespread existence of the female sexual pathologies, basic science has chosen to focus the bulk of its research on male erectile dysfunction. Over the past 15 years there has been a drastic increase in the understanding of the anatomy and physiology as well as pathophysiology behind the male erection (20-25). There have been advances in the diagnosis and treatment of the many male sexual syndromes including impotence. Over the same period of time there has been a serious absence of research focusing on the pathophysiology of female sexual dysfunction, its causes and potential treatments. While there are numerous reasons for the apparent lack of interest in FSD research, only 3 explanations have been postulated (19). Some believe that the strong taboo against female sexuality in our society has hindered laboratory research of the subject. There has also been a serious lack of support from the large funding

agencies, which is only now turning around. There has been a lack of a well-defined, inexpensive model for the study of FSD. What this leaves us with is a lack of an established animal model of female sexual arousal that can be used by researchers involved in the current increase in FSD research. I have proposed the female rat as a model to be used in this research for a variety of reasons.

9.2 Why The Rat?

Sexual function and dysfunction have been studied in a variety of organisms. Studies have been performed examining blood perfusion, velocity and pressure in rabbits, monkeys and dogs to name a few (19,26). These experiments have garnered important data into the functioning of female sexual physiology. However, there are many drawbacks to working with these models. They lack certain characteristics, found in the rat, that are necessary for a complete animal model of sexual arousal.

The majority of these studies have been based upon indirect measurement techniques such as photoplehtysmography, thermal clearance and other temperature-based methods (19,26). As well, working with large animals such as dogs and monkeys can be cost-prohibitive. While these types of studies may be appropriate for larger labs, the majority of researchers are

unable to generate the funds necessary to purchase and maintain these types of animals.

Another important feature in the rat is the wide range of pathological models. These models are well studied and established in the rat and are not always available in other species. Rats can be purchased with genetic manipulations, which allow them to develop a wide range of disease relevant to FSD. These diseases include, but are not limited to, atherosclerosis, diabetes and Parkinson's. These rat models of disease are well established and studied. In addition, the study of the aged animal is much more convenient in the rat as the lifecycle of the rat is considerably shorter than other animals used in similar research. These physical characteristics allow researchers to study female sexual function and arousal in animal models of particular disease states.

While FSD may be a relatively new topic in research, male sexual dysfunction and erectile insufficiency are not. These topics are well studied and have been investigated extensively throughout a variety of species. However, the bulk of impotence and erectile dysfunction research has been performed in male rats. This fact is of utmost importance. Establishing a model of sexual dysfunction in the female rat allows us to compare our data in female rats against a well-

developed and established model in the male of the same species.

9.3 Why Examine The Physiology and Histology?

A properly defined physiological model of sexual arousal in the female rat includes the physiology and neurophysiology behind the lubrication-swelling reaction. Also required are the anatomy, neuroanatomy and histology of the related organs and tissues involved in female sexual arousal. The anatomy and histology of the vulva and sexual organs of the female rat are described in the literature (32). As well, the central neurophysiology of sexual arousal in both the male and female rats has also been elucidated (11). Therefore, all that has been missing from the literature is the physiology and histology of the lubrication-swelling reaction itself. This demonstrates the necessity for direct measurement of the physiology involved in clitoral engorgement. It also demonstrates the necessity to measure what effects SNP, VIP and CGRP, as well as their antagonists, have on the response to electrical stimulation of the clitoral nerves. Lastly, it is necessary to examine the clitoris for the endogenous presence of these neurotransmitters. Direct measurement of clitoral blood velocity using a laser Doppler probe as well as examination of the effect and presence of neurotransmitters has not yet been reported in the female rat to the best of my knowledge.

10.0 Relevant Information Garnered From These Experiments

The present experiments have enabled us to develop a working model of sexual arousal in the female rat. Doing so has allowed us to gain relevant information that will enable further research to continue in this understudied area of interest.

10.1 Experiment #1

Male arousal is characterized by an increase in blood flow to the corpora cavernosa and consequently an erection. Female sexual arousal is also characterized by engorgement of the corpora cavernosa of the clitoris and of the labia as well as transudation of fluid across the vaginal wall. The present model demonstrates the homologous nature of this response between the two sexes. The parasympathetic branch of the nervous system is responsible for the increase in blood velocity and engorgement of the penis, clitoris and vagina during sexual excitation. Results of the first experiment demonstrate that clitoral and vaginal blood velocity can be modulated by electrical stimulation of the dorsal clitoral and pelvic plexus nerves. Stimulation of these nerves results in an increase in blood flow to the respective organ. Our results also infer that our stimulations took place on parasympathetic fibers.

There are very few studies of sexual function in the female rat. However, McKenna *et. al.* have described the role of the central nervous system. They did so by recording vaginal and uterine contractions (reactions indicative of orgasm in the female) following urethral stimulations. These studies would make an interesting cohort to the present study. Together they can shed light on the function of the vasculature and urethrogenital muscles in normal and diseased animals.

Previous investigations have demonstrated the importance of the rat model in erectile function in males (27). Stimulations of the cavernous nerve or dorsal penile arteries have triggered increases in blood flow to the corpora cavernosa, and consequently, erection. This model has shown the same physiological response and will enable the study of female sexual function in the rat.

The results from this experiment prove that blood velocity in the clitoris and vagina is increased upon electrical stimulation of the dorsal clitoral and pelvic plexus nerves. It also shows that laser Doppler flowmetry can record these velocity changes. The female rat can therefore be used as a model in the study of vascular physiology, vascular pharmacology and vascular sexual dysfunction in clitoral and vaginal tissue. Compared to the other available models, the rat should prove interesting and

affordable, two important considerations for the modern research laboratory.

10.2 Experiment #2

A variety of different neurotransmitters have been implicated in erectile physiology. They are Nitric Oxide (NO), Vasoactive Intestinal Peptide (VIP) and Calcitonin-Gene Related peptide (CGRP). Due to the numerous homologies between male and female erectile tissues the possibility that these neurotransmitters can play a role in the lubrication-swelling reaction seen in female arousal has been postulated.

NO has been implicated in the vasodilation of smooth muscle in the corpus cavernosum of the penis (20,21). Nitric Oxide Synthase (NOS) has also been localized within the sexual organs of the human female (28,29). Our results show that the NO-donor, SNP, augments the vasodilatory response to electrical stimulation of the dorsal clitoral nerve. The NOS antagonist L-NAME inhibited the vascular response to stimulation, which resulted in a very small increase in velocity after stimulation was performed. The data garnered from this experiment shows that NO plays a significant role in the clitoral vasodilation that occurs upon stimulation of the dorsal clitoral nerve. This corporal vasodilation is comparable to the response seen in males during similar experiments (24,25). However, L-NAME

infusion did not completely stop vasodilation from occurring upon stimulation. This may imply that NO is not the only neurotransmitter involved in clitoral vasodilation. In addition, a lack of a proper dosage-control study with L-NAME and SNP may have resulted in too low a concentration of the L-NAME. This would not have caused complete antagonism of endogenous NOS.

The second neurotransmitter examined was VIP. VIP is also responsible for the vasodilation causing penile erection (21). VIP also modulates some of the vasodilation that occurs in the female genital tract and has been localized within neurons innervating the vagina and clitoris of human females (30,31). Infusion of VIP in the female rat causes an increase in the vascular response to electrical stimulation of the clitoral nerves. Stimulation after VIP infusion significantly increased the blood velocity to the clitoris compared to stimulation before administering VIP. The amplification caused by VIP was blocked upon infusion of VIP-Cl-Phe^{6}-Leu^{17}, a VIP-antagonist. These data show that in addition to NO, VIP may play a role in the lubrication-swelling response in the female rat. They also point out that a VIP-antagonist can attenuate the effects of VIP. This lends proof to the idea that VIP is responsible for the observed reaction.

The third neurotransmitter examined was CGRP. CGRP is a vasodilator with an accessory role in penile erection (20). It has been localized to the female genitourinary tract. Unlike NO and VIP, CGRP was unable to modulate the response to electrical stimulation. However, when the CGRP antagonist was infused, there was a difference between the agonist infused and the antagonist infused groups. These results may simply be pointing out that CGRP acts as a co-transmitter, secondary to other neurotransmitters involved in clitoral vasodilation. CGRP may not be sufficient on its own for an increase in blood flow and only when inhibited is its effect noted. This second postulation also makes sense as CGRP has been shown to increase the rigidity of an already erect penis (20). CGRP is not sufficient to cause erection on its own.

The histological results of this experiment add further weight to this model. NOS, VIP and CGRP were all found to be localized within the clitoris of the female rat. In addition to the fact that these neurotransmitters have an effect on vascular flow within the clitoris, they are also found there endogenously. This finding adds support to the belief that these neurotransmitters modulate the vasculogenic response to sexual arousal in the normal female rat.

This experiment has added further information to our model of sexual arousal in the female rat. We are now able to attribute the clitoral vasodilation and engorgement associated with arousal to the actions of NO and VIP. These data are also informative because they highlight further similarities in-between the sexual physiology of males and females. This should provide the needed impetus to continue with research in female sexual dysfunction. There have been numerous investigations into the particulars of male sexual physiology. Many of these have been performed in the rat model. These studies should be mirrored and expanded in the female rat in order to provide a proper understanding of the pathophysiology behind FSD.

11.0 Foregoing and Future Research

The entire field of organic female sexual dysfunction is in need of basic laboratory investigation. I have presented a preliminary model of female sexual arousal in the female rat. However, there are many limitations within the techniques used to derive the model as well as the model itself. Work is still needed to fully develop this model.

Expansion of the organs studied must occur for a complete model. In addition to clitoral blood velocity, the effect of neurotransmitters on the vasculature of the internal and external vaginal wall should be examined. Internal vaginal pressure changes could also be measured. The change in pressure in response to both stimulation of the pelvic plexus nerves and neurotransmitter infusion could be examined. Separate histological sections of the distal middle and proximal thirds of the vagina could also be examined for their proprietary neurotransmitter content.

Expansion of the examination of the neurotransmitter must also occur. Infusion must be made more locally. In the present experiment, neurotransmitter infusion was systemic. This resulted in a time delay of the neurotransmitter reaching the selected organs as well as a dilution effect throughout the body. Future experiments should involve infusion much more locally, as has been done in the male, such as the internal pudendal or internal iliac

arteries. It would also prove interesting to re-infuse the agonist after infusion of the antagonist i.e. re-infuse SNP after administering L-NAME. This would demonstrate whether the antagonistic effect can be reversed by adequate agonist infusion. Dosage-response curves would also have to be generated for the infused drugs. Examining the interaction between the different neurotransmitters is also important in establishing a whole model. It would be interesting to infuse an NO blocker followed by VIP and stimulate electrically or perform the reverse, infuse a VIP blocker and infuse with an NO donor followed by an electrical stimulation. This would allow us to examine the inter-relationship between NO, VIP and clitoral engorgement.

It must be strongly emphasized that the vasculogenic aspect is only a portion of the overall sexual response cycle. This model does not account for the behavioral and psychological aspects of arousal. Arousal is easily observed in the male. One is able to equate erection with arousal. However, the female presents a more difficult case. The physiological model must be matched with behavioral studies on sexual arousal in the female rat. Appetitive behaviors, such as pacing, must co-occur with the increase in clitoral and vaginal blood velocity in order to definitively state that these are arousal reactions being observed.

An animal model has been primarily developed that facilitates the investigation of the physiology of female sexual function and the associated pathophysiologies. However, as pointed out, there is still basic research to be done before any definitive studies can occur. If pursued, the rat model should be able to form the basis of the study of FSD in animals and should at the very least prove to be as worthy as its male counterpart.

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