Development and Characterization of a Rat model of Female Sexual Dysfunction

by

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Abstract

Female sexual dysfunction (FSD) is undergoing rapid exploration but there are still few research tools available. The objective of this study is to create and characterize a novel model of sexual function in female rats. The well established model of male erectile function using apomorphine was used as the basis for the development of this animal model of FSD. APO at low doses (80ug/kg s.c.) in male rats induces a characteristic, centrally initiated hormone-dependent sexual response involving a patterned behavioral response followed by an erection.

In this study, the major findings showed that APO can induce a similar centrally initiated patterned behavioral and genital vasocongestive response in female rats. The APO-induced patterned behavioral response was similar to the male in that every characteristic movement before the occurrence of the genital response in the male occurred in the female. In addition, the genital response (erection in male and genital vasocongestion in female) showed similar timing with respect to duration and rapidity of onset. This suggests that the APO-induced genital response in the female rat is analogous to erectile response in the male rat of being a genital vasocongestive arousal (GVA) response.

Further studies demonstrated that this APO-induced GVA response in the female rat was hormonally dependent. Removal of most of the endogenous hormones (through ovariectomy (OVX)) markedly diminished the APO-induced GVA response. While testosterone supplementation 36 hrs prior, restored the response. The role that nitric oxide (NO) plays in the female rat sexual response was also investigated. NO is an important mediator of the erectile response in the male rat. By inducing an environment of NO-

deficiency in the female rat the APO-induced GVA was significantly diminished. However, upon NO supplementation the response was restored. The interplay between NO and hormones was also investigated to determine whether NO could restore the APO-induced GVA response in OVX female rats. However, NO-mimetic supplementation proved unable to restore the response.

These studies demonstrated that APO can centrally initiate both a behavioral and genital vascongestive response. In addition, the importance of endogenous sex hormones and NO on sexual function and the role they play in mediating this response was also demonstrated. The animal model developed in this study can be used to further investigate the roles that hormones, neurogenic and vasculogenic factors play with regard to genital blood flow and vasocongestion. Furthermore, clinical application can be derived from this model, as this is the first conscious animal model that can parallel the physiological endpoint of genital vasocongestion in female sexual arousal in humans.

Statement of Co-Authorship

The following thesis was produced by Rochard K.S. Beharry with the following co-authorship's and assistance:

- Chapter 2: Dr. Michael A. Adams and Dr. Jeremy P.W. Heaton
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...Finally I will like to dedicate this thesis to my family – Mom, Liza and Nicole, you guys have never let me down – Thanks!

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List of Abbreviations

Ach	acetylcholine
APO	apomorphine
cGMP	cyclic guanosine 3'5' monophosphate
cGRP	calcitonin gene related peptide
CNS	central nervous system
D_1	dopamine type 1
D_2	dopamine type 2
ED	erectile dysfunction
FSAD	female sexual arousal disorder
FSD	female sexual dysfunction
GVA	genital vasocongestive arousal
i.v.	intra-venous
L-NAME	N-nitro-L-arginine methyl ester
MPOA	medial preoptic area
MSD	male sexual dysfunction
NANC	non-adrenergic non-cholinergic
NE	norepinephrine
NO	nitric oxide
NOS	nitric oxide synthase
OVX	ovariectomy
nPGi	nucleus paragigantocellularis
PAG	periaqueductal gray
PNS	peripheral nervous system
PVN	paraventricular nucleus
s.c.	subcutaneous
SNP	sodium nitroprusside
SSRI	selective serotonin reuptake inhibitor
VIP	vasoactive intestinal peptide
VMN	ventromedial nucleus

Chapter 1: General Introduction

Preamble

The Focus of this study is to develop a female rat model of female sexual arousal disorder. This disorder is a subcategory of female sexual dysfunction and is characterized by an inability to respond to or maintain an adequate lubricative or swelling (vasocongestive) response during sexual stimulation [1]. In order to develop a sexual response model, a clear understanding of the etiology and physiology of this response must be acquired. Since there is a greater understanding to date of the male sexual response it can be used as a means of comparison to help investigate and understand female sexual function/dysfunction.

1.1 The sexual response

Sexual desire is commonly defined as the broad interest in sexual objects or experiences. Although there is no physiological criterion for desire it is subjectively linked with sexual thoughts, fantasies, dreams or wishes. Associated with sexual desire is sexual arousal, defined using subjective criteria but also by the physiological responses involved (e.g. increased blood flow, genital vasocongestion) [2]. Physiological sexual arousal involving genital vasocongestion occurs in both males and females. This vascular response occurs as a result of vascular smooth muscle relaxation, thereby increasing blood flow into the penis and female genitalia [3] [4].

The penis in the male subsequently becomes engorged with blood (tumescense); a response which is maintained by the blood becoming trapped in the penis via venous occlusion to form the erection [5]. In females vasocongestion occurs in the vagina, vulva, clitoris, labial walls, uterus and possibly the urethra. Along with engorgement of the female genitalia, lubrication arises at the epithelial surface as a result of the increased hydrostatic pressure exerted on the vaginal capillaries [6].

Both the male erection and vasocongestion in female genital arousal, occur via changes in hemodynamics determined by smooth muscle tone in response to neurogenic signaling [7]. Both a penile erection and female genital vasocongestion begin in the central nervous system and are mediated by activation of peripheral nerve traffic. The erection is mediated through changes in the activity in the thoracolumbar sympathetic, lumbrosacral parasympathetic and pelvic somatic nerves [8]. While female genital vasocongestion is mediated via changes in activity in the pelvic parasympathetic, hypogastric and paravertebral sympathetic, pudendal somatic nerve [9] [10] . The sympathetic, parasympathetic and non-adrenergic non-cholinergic (NANC) nerve terminals in the peripheral nervous system have been suggested to account for most of the neural factors involved in the regulation of vasocongestion at the level of the penis and female genitalia [8] [11].

1.2 Sexual Anatomy

Anatomy of the erection

The penis consists of three corpora – the paired corpora cavernosa and corpus spongiosum. A thick fibrous sheath, the tunica albuginea, surrounds each corpus cavernosum. The major blood supply to the penis is via the internal pudendal artery which branches of into other arteries with the paired cavernous artery being the main blood supply for an erection ([11] [12].

In the flaccid state the penis is dominated by sympathetic influences. There is minimal blood flow through the arterioles and the cavernosal smooth muscles are contracted. After sexual stimulation, parasympathetic and NANC nerve activity dominates resulting in relaxation of the arterioles and cavernosal smooth muscles [8] [12]. This relaxation results in increased blood flow and increased intracavernous pressure (filling phase). The increase in intracavernosal pressure compresses and obstructs the plexus of venules against the tunica albuginea causing a decrease in

the venous outflow system [5]. Since arterial inflow is increased and venous outflow is decreased there is a vasocongestive effect created. This vasocongestive effect is commonly known as an erection. In the detumescence phase, increased activity in the sympathetic nervous system leads to increased tone in the arterioles and a decrease in intracavernosal pressure. The venous occlusion induced congestion is removed and venous outflow returns to normal resulting in a flaccid penis {1810}.

Female pelvic anatomy

An understanding of the female pelvic anatomy is essential when trying to evaluate and even treat female sexual dysfunction. The female pelvic anatomy is grouped into two-categories – the internal genitalia and the external genitalia. The internal genitalia consist of the vagina, uterus, fallopian tubes and ovaries. The external genitalia is collectively known as the vulva which consists of the labia, clitoris, interlabial space and vestibular bulbs [13]. During sexual arousal there is increased blood flow to the vagina, clitoris, vestibular bulbs and labial walls.

The wall of the vagina consists of three layers: 1) an inner aglandular mucous membrane epithelium, 2) an intermediary richly supplied vascular muscularis layer and 3) an outer adventitial supportive mesh [14]. Genital vasocongestion and vaginal canal lubrication occurs during sexual arousal as a result of increased blood flow through the vaginal arteries which arise from the uterine, hypogastric, hemorrhodial and clitoral vessels [15]. The increased blood flow causes engorgement of the vaginal wall which causes an increase in pressure that forces plasma out of the subepithelial vascular bed through the vaginal epithelium via intraepithelial spaces into the vaginal canal [16]. In addition to increased blood flow and lubrication the vagina lengthens and dilates during sexual arousal due to smooth muscle relaxation [13]. The clitoris is an erectile

organ similar to the penis and arises embryologically from the same structure, the genital tubercle. The clitoris is comprised of three parts: the outermost glans, the midline corpus (body) and the asociated bilateral crura [17]. During sexual arousal the corporeal bodies of the clitoris become engorged with blood due to increased flow through the clitoral cavernosal arteries, which originate in the iliohypogastric pudendal arterial bed [18]. The vestibular bulbs are also considered erectile organs in the female and they too become engorged upon sexual arousal. However, unlike the penis there is no subalbugineal layer between the erectile tissue and the tunica albuginea layer. In the male there is a venous plexus that expands and is compressed against the tunica albuginea reducing venous outflow and thus causing a rigid erection. Therefore the absence of this structure suggests that the vagina, clitoris and vestibular bulbs achieve tumescence but not rigidity [13].

1.3 Neurophysiological mechanisms of sexual response

In order to elucidate the etiology of the sexual response cycle in both males and females the physiology of the pathways regulating this response from central initiation to peripheral completion must be understood. The male sexual response has been thoroughly investigated and there is a firm understanding of CNS, PNS and peripheral neurotransmission involvement in this response. There is not such a complete understanding in the female sexual response, however mechanisms into this response at all physiological levels are being rapidly explored. A critical observation that is becoming more clear as research progresses is the similarities in pathways at all levels between the male and female response.

Male sexual response – erection

A. Central nervous system

In the CNS there is interaction among different segments of the brain to produce the complete sexual response via the spinal reflex system. Brainstem structures such as the serotonergic nucleus paragigantocellularis (nPGi) are sites of major inhibitory activity for the sexual response cycle. Neurons from the nPGi project directly onto the spinal cord and help coordinate an inhibitory signal [19]. The paraventricular nucleus (PVN) of the hypothalamus has been shown to be a major player in excitatory control of sexual response, especially the erectile response in the male penis. It projects both directly and indirectly via other CNS components onto spinal cord pathways [20]. Unlike the nPGi which relay a tonic inhibitory signal, the PVN is probably only activated when sexual desire or a sexual situation arises [21].

Desire, motivation and the ability to interpret sexual situations in order to demonstrate sexual behavior is thought to be processed in higher region of the brain such as in the amygdala of the forebrain and the medial preoptic area (MPOA) of the hypothalamus [22]. Signals from these areas are not directly projected onto the spinal cord but are relayed via the brainstem and other brain regions such as the periaqueductal gray (PAG) of the midbrain which act as a relay system for sexually relevant stimuli between the higher brain regions and the lower brain/spinal cord areas [2] [23] [24].

B. Peripheral nervous system

A normal erection requires input from three different parts of the peripheral nervous system - thoracolumbar sympathetic, lumbosacral parasympathetic and pelvic somatic. [25]. In the periphery, the pelvic nerve ganglion controls activity in many pelvic organs. It receives input

from both the pelvic nerve and the hypogastric nerve. Of the many postganglionic nerves exiting the pelvic nerve ganglion the cavernous nerve innervates the penis [26]. The cavernous nerve contains both sympathetic and parasympathetic fibres. Sympathetic stimulation, via the thoracolumbar sympathetic nerve, causes detumescence of the penis by inducing vasoconstriction in the penile vasculature. Parasympathetic stimulation, via the lumbosacral parasympathetic causes tumescence of the penis by inducing vasodilation in the penile blood vessels and increasing blood flow to the cavernous tissue [9]. In addition to receiving autonomic signaling, the penis also receives somatic input. While autonomic innervation is responsible for psychogenic erections the somatic innervation is generally accepted to be responsible for reflexogenic erections. These reflexogenic erections occur via sensory pathways from the penis, i.e. the pudendal nerve, and can be evoked by manipulation of the penis [12].

C. Peripheral neurotransmission

At the level of the penis the neural control mechanisms for an erection are typically described under the three headings – adrenergic, cholinergic and non-adrenergic non-cholinergic (NANC) [8].

Parasympathetic stimulation and the consequent release of acetylcholine (Ach) has been shown to cause vasodilation of the penile vasculature and subsequent tumescense. However, evidence has shown that Ach on its own is not very effective in causing vasodilation of penile tissue. The role of the cholinergic nerves therefore has been suggested to be more of a mediator rather than a primary factor in causing erections [27].

In recent times there has been a shift in attention from cholinergic factors to NANC factors in causing penile erections. Nitric oxide (NO) is a potent vasodilator and has been shown to play a

crucial role in the mediation of erections. Studies have demonstrated that blockade of nitric oxide synthase (NOS), the enzyme responsible for the synthesis of nitric oxide, diminishes or may even completely obliterate the erectile response [28]. However, other NANC factors such as vasoactive intestinal peptide (VIP) and calcitonin gene related peptide (cGRP) have been implicated in causing vasodilation at the level of the penis [29]. Detumescense of the penis is attributed to increased sympathetic outflow. Release of neural factors such as norepinephrine (NE) from adrenergic nerve terminals have been shown to cause vasoconstriction in the penile vasculature and appears to be the major factor in keeping the smooth muscle of the penile vasculature in a contracted state [12].

Female sexual response – genital vasocongestion

A. Central nervous system

There is limited understanding of central nervous system control on the female sexual response. Different sites are thought to play particular roles with numerous interactions occurring between different brain regions to complete the total sexual response cycle. However, similar roles are thought to exist between similar brain regions for male and female. Higher brain centres such as the amygdala of the forebrain have been associated with the sexual response, although its specific role has not been elucidated. It has been suggested that the amygdala plays a role similar to that in the male, as a mediator of sexual desire and motivation [30]. Hypothalamic structures such as MPOA, ventromedial nucleus (VMN) and the PVN have been associated with sexual behavior and mate selection [31]. In particular, sexual responses at the genital level (in both male and female), including clitoral and vaginal engorgement in the female, are thought to originate in the PVN of the hypothalamus [9]. The PVN projects directly onto autonomic outflow at multiple

segments as well as onto pelvic autonomic and somatic efferents in the periphery [32], which are responsible for increased genital blood flow. Female genital vasocongestion are mediated through peripheral nerves in the lumbosacral region that are mediated through projections of oxytocin neurons from the PVN. Further, oxytocin is released from the PVN upon sexual arousal and orgasm [33]. In addition, electrical stimulation of the PVN as well as direct oxytocin application onto CNS areas have been shown to cause penile erections [34] [35]. Thus, signaling from these areas appear to either directly project onto the spinal cord, or be relayed via the brainstem and other brain regions such as the PAG of the midbrain. The PAG acts as a relay system for sexually relevant stimuli between the higher brain regions (e.g. amygdala and hypothalamus) and the lower brain / spinal cord areas [2] [23] [24].

B. Peripheral nervous system

The female genitalia receives both autonomic (sympathetic / parasympathetic) and somatic innervation. Autonomic innervation originates in two plexi – the hypogastric plexus and the sacral plexus. The fibers from these plexi join together to form the uterovaginal plexus. The uterovaginal nerves, which carry both sympathetic and parasympathetic fibers, innervate the vagina, clitoris, vestibular bulbs and labial walls. Somatic innervation in the clitoris and vagina travels through the pudendal nerve to the sacral region of the spinal cord [36].

C. Peripheral neurotransmission

It has been hypothesized that the neurogenic and endothelial-mediated release of the NANC factor nitric oxide (NO) serves as the primary mediator in smooth muscle relaxation leading to female genital engorgement. NO donors have been shown to enhance smooth relaxation in rabbit

clitoral cavernosal strips *in vitro* [30]. One mechanism by which genital engorgement occurs is via activation of guanylyl cyclase to produce the 2nd messenger cyclic guanosine 3'5' monophosphate (cGMP), which causes smooth muscle relaxation [37]. NO donors have been shown to increase cGMP production in both human and rabbit vaginal smooth muscle cell cultures [38]. Another NANC factor – vasoactive intestinal peptide (VIP) has been implicated as one of the leading mediators in female sexual arousal. Similar to NO, VIP has been shown to cause relaxation in vaginal smooth muscle tissue [39]. In addition, intravenous infusion of VIP caused an increase in genital blood flow and vaginal lubrication [40] [4]. The precise role that the adrenergic / sympathetic and cholinergic / parasympathetic systems play in regulating blood flow to the female genital area has not been investigated as extensively as in male erectile function. A number of studies have suggested that, similar to the male, the parasympathetic nervous system through neurotransmitters such as acetylcholine (Ach) plays a more facilitatory role in female genital sexual arousal response while the sympathetic nervous system plays a more inhibitory one [9]. Sympathetic activation, via adrenergic agonists, has been shown to decrease vaginal blood flow [41]. Whereas sympathetic inhibition, by adrenergic antagonists has demonstrated a positive effect in the female sexual arousal response [42].

1.4 Impact of hormones on sexual function

In both male and female, endogenous sex hormones play an essential role in the maintenance of libido [43] and physiological sexual arousal (genital vasocongestion) [44]. From the higher centres in the CNS through the PNS to the peripheral vasculature there exist hormone receptors which play a role in regulating the sexual response. Neurons within the midbrain, hypothalamus and amygdala contain gonadal hormone receptors that are likely sites where hormones can regulate

sexual desire and motivation [21]. Sexual reflexes via the spinal cord also appear to be regulated by hormones. For example, behavioral studies have shown that sexual reflexes in spinal cord transected rats were influenced by androgen action on the spinal cord [45]. Hormones also play an important role in maintaining the structures of the sexual organs. Research by Berman *et al*, demonstrated the importance of estrogen in maintaining vaginal mucosal integrity and vaginal blood flow [46]. Traditionally, testosterone appeared to be the dominant hormone in regulating male sexual function while estrogen assumed that role in females. However, even though estrogen is still considered the primary hormone involved in the maintenance and functioning of the female sexual response pathway, testosterone in recent times has also been implicated in the dysfunction of this pathway.

Testosterone

Testosterone has long been recognized to be an important factor for sexual well being in males. Studies have demonstrated that withdrawal of exogenous testosterone from hypogonadal or castrated men causes a rapid decrease in sexual interest. However, in these same individuals sexual interest is reestablished upon testosterone supplementation [47]. Removal of endogenous testosterone can also inhibit the erectile response. Studies have shown that castration almost obliterates the erectile response in castrated rats. However, upon exogenous testosterone supplementation the erectile response was restored to normal levels [48] [49]. In females, lower than normal testosterone levels have been suggested to be linked to a decline in both sexual desire and physiological arousal (genital vasocongestion) along with vaginal atrophy [50]. Thus, testosterone supplementation has been shown to enhance subjective arousal in both pre- and postmenopausal women who suffer from inhibited sexual desire [51] [52].

Estrogen

Estrogen plays a minimal role in regulating sexual function in males. However estrogen is a critical factor in the maintenance of sexual organs in the females. Estrogen also appears to act as a vasoprotective and vasodilatory mediator in the female genitalia. A decrease in estrogen levels by ovariectomy or following the onset of menopause causes thinning of the vaginal walls via cellular apoptosis [53] [46]. Estrogen has also been shown to be a mediator of blood flow in the female genitalia by regulating the production of NOS in the vagina and clitoris [46]. These observations may account for the decrease in sexual responsiveness and physiological sexual arousal along with dyspareunia that postmenopausal women experience [2]. The influence estrogen has on sexual desire is unclear. Some studies have suggested that it plays a minimal role in sexual desire in women. Evidence for this is seen in women exhibiting a low sexual drive, where estrogen treatment alone does very little to enhance desire. There is a general agreement that women experience a loss of sexual desire in the postmenopausal stage but the decline in estrogen can not be convincingly attributed to this condition [54]

1.5 Female sexual dysfunction – classifying and evaluating

Investigations into the mechanisms and etiology of female sexual dysfunction (FSD) have lagged behind the male counterpart, although there appears to be a higher prevalence of sexual dysfunction in females than in males (MSD) [55]. It is not surprising then that pharmacotherapy available for FSD also lags behind treatment of the male condition. A major obstacle involved in evaluating FSD is that it is more difficult to diagnose than is the male dysfunction. With regard to male sexual function there exists well-established endpoints, such as erectile function.

However, FSD appears to involve a more complex association of factors – some physiological and some psychological (many of which can only be subjectively assessed). This has made the devising of clinical studies and possible therapeutics more challenging.

In recent times, attempts have been made to define and characterize FSD. The American Psychiatric Association has established the most widely accepted definition and characterization of this disorder [56]. FSD is currently defined as disturbances in the processes that characterize the sexual response cycle or by pain associated with sexual intercourse. In addition it has been classified into 4 sub-categories: 1) sexual desire disorders, namely hypoactive sexual desire or sexual aversion disorders; 2) sexual arousal disorders 3) orgasmic disorders; and 4) sexual pain disorders, which include dyspareunia and vaginismus [56]

Sexual desire disorders

Sexual desire disorders, namely hypoactive sexual desire or sexual aversion disorders involve an overall disinterest in sex [1]. There are many factors that can decrease sexual desire. Negative psychological factors, (e.g. sexual abuse [55], antidepressant medication (e.g. selective serotonin reuptake inhibitors (SSRI) [42] and decreased hormonal levels [57] have all been implicated as playing a role in decreased sexual desire. Despite this, desire disorders are very difficult to diagnose because there are no physical or physiological markers that have been delineated to characterize this problem. At present, the affected individuals make diagnoses on self-assessment. Such diagnoses may be difficult to categorize and treat because of the inherent variability and subjectivity from individual to individual. Since there are many etiologic determinants in female hypoactive sexual disorder therapeutic interventions have varied widely [58]. Attempts at treatments have included hormonal supplementation (estrogen + testosterone

supplementation), pharmacological (vasodilatory agents) and psychological interventions [57] [59] [60].

Sexual arousal disorders

Sexual arousal disorder involves an inability to respond to or maintain an adequate lubricative or swelling (vasocongestive) response during sexual stimulation [1]. Female sexual arousal disorders (FSAD) have been linked with impairment of physiological processes, such as inadequate blood flow, thereby altering the capacity for vasocongestive and lubricative events in the genitalia [7]. Other factors such as altered hormonal milieu, pelvic injury from hysterectomy, and use of medication such as SSRI have all been associated with FSAD [61]. There are also many disease processes and risk factors involved in FSAD that are common to male erectile dysfunction. These include aging, atherosclerotic vascular disease, hypertension, cigarette smoking and hyperchlosterolemia [30].

There are a number of therapeutics currently being tried and / or investigated to combat this disorder. Hormonal therapy has proven somewhat useful against FSAD. Treatment includes testosterone supplementation alone or in combination with estrogen. However, hormonal treatment involving testosterone has shown varying results and may include potential side effects such as weight gain, hypercholesterolemia and increased facial hair [13]. Alternatively, oral vasodilators such as sildenafil, L-arginine, apomorphine and phentolamine are also currently being investigated and have been suggested to enhance vaginal blood flow in individuals with FSAD. However, factors such as dose, time course of delivery and duration of action are not well understood and need to be addressed further [30] [3] [62].

Orgasmic disorders

Orgasmic disorder is defined as the persistent inability to achieve orgasm after sufficient sexual stimuli and arousal. This type of FSD may have a physiological basis such as hormone deficiencies or pelvic injury through surgery. Psychological factors such as trauma or sexual abuse have also been shown to contribute to this condition [13]

Sexual pain disorders

Sexual pain disorders, are most commonly associated with the condition of pain in the genital area during or after sexual intercourse (dyspareunia) [63]. This disorder can have both a psychological and physiological cause. Usually the psychological cause has to be ruled out before the condition is deemed to be a result of a physiological process [1]. Such psychological issues include phobic reactions, anxiety conflicts, hostility (towards partner) or sexual aversion [64]. However the dyspareunia may be a direct result of some disease or conditions. Dyspareunia has shown to be secondary to such conditions as - vaginismus (involuntary spasms of the introital muscles), vulvodynia, vulvar vestibulitis or inadequate lubrication [65].

Interrelation among sexual disorders

An important understanding of the condition of FSD is that there is not normally a single subcategory that typifies the disorder in any particular individual. For example, hypoactive sexual desire or sexual aversion disorder may be the result of psychological trauma, such as childhood sexual abuse. But this disorder may also be secondary to another disorder such as orgasmic disorder or sexual arousal disorder [1]. Therefore, it is not surprising that when

developing therapeutics for these individual disorders of FSD there will be substantial overlap among treatment options. An ideal approach in treating FSD is to assess both psychological and physiological factors before making any definite diagnosis. Previously, psychological treatment has proven efficacious in individuals with sexual aversion disorder and sexual pain disorder where some sort of psychological trauma may have caused the onset of the specific disorder [64] [60]. However this type of treatment has not proven helpful in FSAD. This suggests that a more physiological approach should be taken when trying to treat FSAD because of the obvious physiological problems such as lack of genital vasocongestion associated with this specific type of FSD.

1.6 Animal models of female sexual function/dysfunction

Animal models have almost always been the initial step in basic scientific research. For example, in sexual research the erectile model has been long used as the standard for measuring the male sexual response. Since this response can be identified in both male humans and animals such as rats, animal models investigating erectile function can be used as a means by which sexual function / dysfunction can be evaluated. Animal models of erectile function / dysfunction have proven to be of great importance when evaluating etiology, pathophysiology and therapeutics of this disorder. However, with regard to female animal sexual function research, there has been a relative lack of research done in developing models compared to the male animals. One of the reasons for such an inadequacy in investigating female sexual function / dysfunction is that fewer observable parallels can be drawn between the sexual responses of female rats and women than between the sexual responses of male rats and men.

Up until recently, there was thought to be no physiological observable endpoint to the sexual response in females that could be paralleled in an animal model. As a result, most of the animal models developed to investigate the etiology and pathophysiology of female sexual dysfunction were behavioral in nature. Sexual receptivity has generally been measured in the female rat by observing the lordosis to mount ratio [66] [67] [68] [69] [70]. Lordosis occurs in the female, and is described as planting of the forepaws, elevation of the head and arching the back, which elevates and exposes the pudendal region. Mounting occurs in the male and is described as an approach from the rear of the female, associated with rapid pelvic thrusting and palpation of the female's flanks [68]. Thus, the characterization has been that a higher number of lordotic responses to a fixed amount of mounts indicate an increased sexual receptivity of the rat.

An important criticism regarding the use of lordotic responses as an indicator of sexual response is that the rat is perceived to be sexually receptive on a behavioral event alone when an essential part of the female sexual response has been attributed to increased blood flow to the genital area. Recently, Park *et al* developed a female animal model of electrostimulation that demonstrated sexual arousal was achieved by increased blood flow and subsequent engorgement of the genital region [7]. Therefore it is likely that both behavioral and physiological hemodynamic processes occur concomitantly in the rat to signal a sexual response. This response is seen in the male rat when apomorphine (APO) is used to induce an erectile response. That is the response involves a patterned behavioral response that precedes a physiological response (erection) [71]. APO elicits erections by acting on the dopamine type 2 (D_2) receptors of the PVN of the hypothalamus [72].

A pilot study conducted by Beharry *et al* investigated the effect NO deficiency via NOS blockade had on the APO-induced erectile response in male rats. The study's primary

observations demonstrated that the APO-induced erectile response was reduced in a NO deficient rat. But, in addition, a key observation was that the rat demonstrated the patterned behavioral response associated with the physiological response of the erection on occasion but did not have the erection. In this study, even though the behavioral response still occurred, since no physiological relevant response (i.e. erection) was observed, a sexual response was deemed not to have happened.

The findings suggest that a behavioral response alone (i.e. lordosis) is not a sufficient determinant (on its own) to establish whether a sexual response has occurred or not. For example, in a study performed by La Vaque *et al* [68], sexual receptivity was determined in female rats after large electrolytic lesions were made in the hypothalamus. The sexual receptivity of the female rats using the lordosis to mount ratio method, was unchanged both before and after the lesioning occurred because the behavioral response was unchanged [68]. Studies in the following years have demonstrated the importance of the hypothalamus in controlling blood flow associated with the sexual response [73]. This study clearly indicates the shortcomings of the traditionally used method of lordosis to mount ratio in quantitatively measuring female sexual response. By only considering the behavioral response as the sole indicator of sexual response it is difficult to determine if a physiological response (increased blood flow to genitalia) has occurred or not.

An animal model that can both assess both behavioral and physiological aspects of the female sexual response would be more helpful in understanding FSD. In addition, an observable physiological endpoint such as increased blood flow to the genitalia would be of added benefit because this animal model may be used to parallel the prevalent FSD of female sexual arousal disorder in which blood flow to the genitalia is the primary issue.

1.7 Apomorphine model of erectile function

A number of different animal models for male sexual function that have been developed. These models have used varying techniques including pharmacological induction of tissue responses, electrostimulation and copulation studies to investigate causes and possible therapeutics for male sexual dysfunction. Of these the pharmacological rat model of apomorphine (APO)-induced erections, in our hands, seems to be the most reliable technique for a number of reasons. It is non-invasive (unlike electrostimulation) therefore, it can be used in conscious animals in conditions which may mimic those similar to ones that may cause erectile dysfunction (e.g. stress, hypertension, vascular disease) in humans.

The APO-induced erectile model, initially developed by *Heaton et al*, is a well-established model of sexual function in the male rat [74]. As indicated earlier, APO elicits erections by acting on the D_2 receptors of the PVN. The APO-induced erection is similar to a physiological erection in that the same neural pathways are activated, making the APO-induced erection model a useful paradigm to induce erections that involve the entire erectogenic pathway [75]. Given the similarities between an APO-induced erection and a 'natural' erection, this model has proven to be helpful in developing therapeutics for erectile dysfunction. Recent clinical trials have demonstrated the effectiveness of APO in treating erectile dysfunction (ED). Almost 60% of males suffering from ED experienced erections within 25 minutes of sublingual APO administration [76] [77].

The APO-induced response, in the male rat, involves both a patterned behavioral response and physiological (vascular) change in its genitalia [71]. Preliminary studies have shown that APO (a dopamine agonist) also causes an analogous pattern of behavioral response in the female rat

comparable to that of the male. Further support is found in a recent study by Tarcan *et al*, vaginal and clitoral engorgement in female New Zealand white rabbits was achieved following electric stimulation of the vaginal / clitoral pelvic nerve. Administration of APO at low doses improved nerve stimulated vaginal and clitoral engorgement by increasing clitoral intracavernosal pressure and vaginal wall arterial inflow [78].

1.8 Research objectives and hypotheses

An overall objective of this thesis was to determine whether apomorphine at low dose (APO) can initiate a behavioral and physiological genital response in female rats characteristic of a sexual arousal response. Given the similarities observed in APO-induced behavioral responses seen in male and female rats from preliminary studies, we hypothesized that APO will initiate a similar vascular response in female rats involving genital vasocongestion – a critical component of the sexual arousal response [4] [3].

Furthermore, given the dependence of the APO-induced erectile response on endogenous testosterone [35] [49] we determined whether the APO-induced sexual response in female rats is also hormonally dependent. Given the importance of endogenous hormones in maintaining the normal female sexual response we hypothesized that the female response is also dependent on endogenous hormones. To determine, at least in part, which hormones are involved we characterized the effects of exogenous testosterone administration in ovariectomized female rats with regard to the APO-induced sexual response.

Finally, to determine whether nitric oxide (NO) is a key mediator in the female rat sexual response cycle we assessed the effects of inhibiting NO production. Given its importance in regulating the erectile response in the male rat, [28] and its implication in the female sexual

arousal response in mediating blood flow, [27] we hypothesized that NO is a necessary factor for completion of the sexual arousal response in female rats.

Chapter 2: Evidence for centrally initiated genital vasocongestive engorgement in the female rat: Findings from a new model of female sexual behavior (FSB)

2.1 Introduction

Female sexual function is a field undergoing rapid exploration yet the physiological and psychological mechanisms involved remain largely unresolved. As it was in the research on male erectile function & dysfunction, the need for developing animal models, in order to study basic physiology, pathophysiology, and possible treatments of female sexual function/dysfunction has become increasingly important. To date, as would be expected, no one model has been acknowledged to meet the needs of researchers interested in female sexual dysfunction (FSD).

FSD is classified as a condition in which there are single or multiple processes involved in the female sexual response cycle or by pain associated with sexual intercourse [1]. FSD can be categorized into four main areas: 1) sexual desire disorders, namely hypoactive sexual desire or sexual aversion disorder 2) sexual arousal disorders 3) orgasmic disorders and 4) sexual pain disorders which include dyspaureunia and vaginismus [56]. Recent studies suggests that at least 43% [55] of women suffer with some form of dysfunction.

It appears that the dominant sub-category of FSD is female sexual arousal disorder (FSAD) which affects up to 75% of women diagnosed with FSD. Female sexual arousal results in a series of vasocongestive and lubricative events resulting primarily from increased blood flow to clitoral, labial and vaginal tissue [62] [4]. This increased blood flow to the genital area is similar to the male sexual response of the erection. Peripheral innervation of the female genital tract is connected to the CNS in a manner which is anatomically similar to the male [9]. At the level of the female genitalia it appears that the sympathetic, parasympathetic and non-adrenergic non-cholinergic (NANC) systems are responsible for the primary neural regulation of blood flow. The precise contribution (facilitory/inhibitory) of the cholinergic (parasympathetic) and

adrenergic (sympathetic) systems has not been elucidated. However research assessing NANC factors such as nitric oxide (NO), vasoactive intestinal peptide (VIP), calcitonin gene related peptide (cGRP) have all been implicated as factors responsible for increasing blood flow to the genital areas [3] [40] [4].

There have been numerous suggestions regarding the main etiologies of female sexual arousal disorders (FSAD). Including vascular/endothelial [7] [3] [79], neurological [62] and hormonal disorders. Unfortunately therapeutic efforts to address these problems have been met with less success than had been hoped. For example, treatment that has been successful in treating male erectile dysfunction (MED), such as sildenafil (Viagra ®) has shown variable success in combating FSAD [80]. It may be that a different spectrum of activities is needed when devising treatments for women, compared with the approach taken with males, such as dose of drug, duration of action and time course of delivery. In particular, an understanding of the hormonal variations throughout the reproductive cycle in females must be factored in when developing possible therapeutics. This parameter of varying hormonal milieu is not a major factor in the male sexual response to therapy, as testosterone levels remain fairly constant within an individual (at least over the short term).

Male erectile dysfunction (MED) is now a well-characterized field in both clinical and basic research. Decades of investigation have markedly enhanced the diagnosis of the condition and its therapeutics. In this process there has been a very important role for animal models. There have been attempts to develop animal models of the female sexual response, although for the most part, they have been behavioral paradigms. Previous studies have assessed sexual receptivity of the female animal based on a behavioral response. This response involves lordotic posturing (in which the female rat demonstrates concave back flexion, lateral tail deviation and neck extension

[81] in relation to male copulatory contact [69] [82] [81]. Thus according to these studies the female rat when expressing these behavioral characteristics is expressing a sexual response. However other studies have demonstrated that these behavioral responses persist despite the presence of electrolytic lesions in the hypothalamus [68] – a key central site for initiation of peripheral neurogenic signaling [83] [84]. It is very likely that the female behavioral responses do not have to include the peripheral manifestation (ie. increased blood flow to the genitalia) during sexual arousal. In fact a peripheral sexual dysfunction, at the level of pudendal vasculature, may not be detected using these behavioral paradigms alone.

The erection is the obvious, standard response in determining sexual arousal in the human male. In this regard, the apomorphine (APO)-induced bioassay of erectile function in the male rat [74] provides an established animal model of this phase of the sexual response. Apomorphine elicits erections by acting on the dopamine type 2 (D_2) receptors of the paraventricular nucleus (PVN) [72]. This APO-induced response, in the male, involves both a patterned behavioral response and physiological (vascular) change in its genitalia [71]. Preliminary studies have shown that APO (a dopamine agonist) can cause an analogous pattern of behavioral response in the female rat comparable to that of the male [85].

The present study was to characterize both the behavioral and vascular events that occur in the female rat as a result of APO administration. By comparing the components of the APO-induced response in female rat to the APO-induced response in the male rat a better understanding of sexual arousal in the female rat can be achieved. In addition, by administering APO at different points in the estrous cycle of the rat and in an ovariectomized animal we can observe the effect of different hormonal conditions on APO induced sexual response.

2.2 Methods & Materials

Animals

8 adult female Wistar rats (225-250g) obtained from the Charles River Laboratories (Montreal, Canada) were used for the purpose of this study. Animals were housed in polypropylene shoebox cages in a climate-controlled room with a 12/12-hour light/dark cycle. Food (Purina rat chow ®) and water were provided *ad libitum* except during times of testing. The procedures carried out in this study were in accordance with the Canadian Council of Animal care. Rats were handled for 5 consecutive days prior to experimentation to allow for acclimation to the investigator. Prior to experimentation, all rats showed a consistent 4-day estrous cycle consisting of normal periods of proestrus, estrus, metestrus and diestrus.

Experimental Procedure

Modifications to the APO 80 ug/kg protocol originally published by *Heaton et al* (1991) were used for the purpose of this study [74]. Previous dose response assessments (APO – 40, 80 & 120 ug/kg) demonstrated that APO 80 ug/kg, similar to the male, elicited the greatest number of genital grooming responses in the female rat. In brief, APO 80 ug/ml was prepared along with 100 ug/ml of ascorbic acid. Rats were placed in hanging cages with bottoms constructed of Plexiglas®, in a dark, quiet room where they were allowed to acclimate for 10 minutes before the testing period. APO 80 ug/kg was injected subcutaneously (s.c.). Subsequent genital responses and associated yawning responses were observed and recorded for a 30 minute period via a video monitoring system in an adjacent room. A genital response was counted when there was a change in the appearance of the external genitalia accompanied by oral grooming of the

genital area. A yawn was counted as an involuntary opening of the mouth with the appropriate respiratory movement.

Vaginal Smears

Vaginal saline smears from the rat were analyzed under a light microscope to determine the stage of the estrous cycle. The type of cell identified in the smears was dependent on the estrous stage [86] [87] [88] - a) proestrus smear contains nucleated and very few leukocytes.

b) Estrus smear contains moderate number of large cornified squamous cells.

c) Metestrus smear contains many large cornified squamous and polymorphonuclear leukocytes.

d) Diestrus smear contains small epithelial cells and many leukocytes

Experiment A – APO induced responses in intact rats

APO induced genital responses and yawning responses were characterized then determined during each stage of the estrous cycle – proestrus (~12 hr), estrus (~12 hr), metestrus (~21 hr), early diestrus and late diestrus (~57 hr). Stages of the estrous cycle were determined by analyzing the epithelial cell type in vaginal saline smears [86] [87] [88]. (Diestrus lasts about ~57 hrs [87], therefore early diestrus was the considered the 1st half and late diestrus the 2nd half of this segment of the estrous cycle.

Experiment B – APO induced responses in ovariectomized rats

The APO response was determined in ovariectomized (OVX) rats using methods to the above. OVX animals were used to ensure that endogenous hormones, such as estrogen and progesterone, were present in the body at minimal levels. In Brief, OVX were carried out using a

similar procedure to Wayforth *et al* [89]. Animals were anaesthetized with isoflurane gas delivered via a Bain rebreathing system. A small midline dorsal skin incision was made approximately half way between the middle of the back and the base of the tail. Both ovaries were removed by ligating and severing the junction between the fallopian tube and uterine horn. Post-operative analgesia (Buprenex, dose of 0.05 mg/kg of body weight and concentration of 0.3 mg/ml) and the antibiotic Tribrissen 24% (Schering Canada inc.) were administered daily (0.1ml/100 g of body weight) for 5 days. Animals were given at least 1 week recovery before the onset of experimentation.

Data analysis

GVA responses

Statistical analysis between – 1) APO-treated and saline control groups 2) intact and OVX groups to determine statistical difference was performed using Mann Whitney Rank sum test ($p<0.05$). Analysis of each treatment group (APO, saline) to determine statistical difference among different stages of the estrous cycle was performed using the Friedman repeated measures ANOVA on ranks.

Yawning response

Statistical analysis between in APO-treated intact and OVX groups to determine statistical difference was performed using student's t-test ($p<0.05$). Analysis of APO treatment group to determine statistical difference among different stages of the estrous cycle was performed using one way repeated measures ANOVA.

2.3 Results

Characterization of vascular and behavioral responses in female wistar rats to saline and apomorphine (APO)

Upon careful observation, APO (80 ug/kg) administration caused a distinct change in the morphology of the external genitalia of the female rat. This change involved a significant enlargement or engorgement of the genitalia that lasted about 3 seconds. This enlargement of the genitalia can be best described as genital vasocongestive arousal – GVA (figure2-1,lower panel). In addition, the APO-induced GVA response was preceded by a patterned behavioral response. This patterned behavioral response is characterized by a 'startle' response in which the rat's awareness of its surroundings is suddenly heightened. The 'startle' response is followed by a concavity of the back, rearing, in which the rat demonstrates an erect posture by standing on its hind legs and vasocongestion of the external genitalia. Consequently the rat rapidly descends head first into the genital area, which by now is fully engorged and enlarged (GVA), and proceeds to terminate this behavioral response with genital grooming. This behavioral pattern is reminiscent of the male rat response [71].

Genital grooming is also seen in rats without any of the accompanying vascular or a patterned behavioral response (non-GVA) (figure 2-1,upper panel). This non-GVA response was seen in both control and APO tested animals and appears unrelated to the specific sexually or hormonally based responses sought here.

APO and vehicle administration in intact rats

APO was administered to 8 female wistar rats (250-275g) in each stage of the estrous cycle and the number of responses was counted in the subsequent 30 minutes. The average number of responses in each stage was calculated.

There was a significant increase in the average number of GVA responses in every segment of the estrous cycle (peaking in estrus stage) vs. control (figure 2-2, upper panel) – proestrus 1.5±1.06 vs. 0.13±0.35, estrus 1.75±1.67 vs. 0, metestrus 1.5±1.20 vs. 0.13±0.35, diestrus I 1.13±1.36 vs. 0 and diestrus II 0.88±0.85 vs. 0.13±0.35. There was no significant difference in non-GVA responses between APO and control tested animals in any stage of the estrous cycle (figure 2-2, lower panel).

Corresponding yawning responses in APO tested animals was observed in all stages of the estrous cycle in intact animals (2±2.50 in diestrus I to 3.63±3.96 in metestrus (figure 2-3). There was no significant in yawning response among all stages of the estrous cycle. No yawns were observed in control animals.

The GVA responses during all stages of the estrous cycles counted in APO treated rats was almost 20 times as frequent compared to controls (figure 2-4). However there was no significant difference between control and APO tested animals for non-GVA responses (17 vs. 10) (figure 2-4).

The time course distribution of APO-induced GVA responses over the 30-minute period showed GVA responses peaking in the 5-10 time period with 80% of total GVA responses occurring within the first 20 minute time period (figure 2-5, upper panel). The time course distribution of APO-induced yawning responses over the 30-minute period showed the yawning responses peaking in the 15-20 min. time period (figure 2-5, lower panel).

APO and vehicle administration in ovariectomized (OVX) rats

APO-induced GVA responses were determined in 8 OVX wistar rats and the number of responses for each rat in the subsequent 30 minutes was counted. There was a significant decrease in APO-induced GVA responses observed in OVX versus intact of approximately 80% (figure 2-6, upper panel). There was no significant difference in non-GVA responses between OVX versus intact animals in both APO and control tested animals (figure 2-6, upper panel & lower panel). APO induced yawns were observed in both OVX (3±2.50) and intact (2.9±2.68) animals (figure 2-6, lower panel).

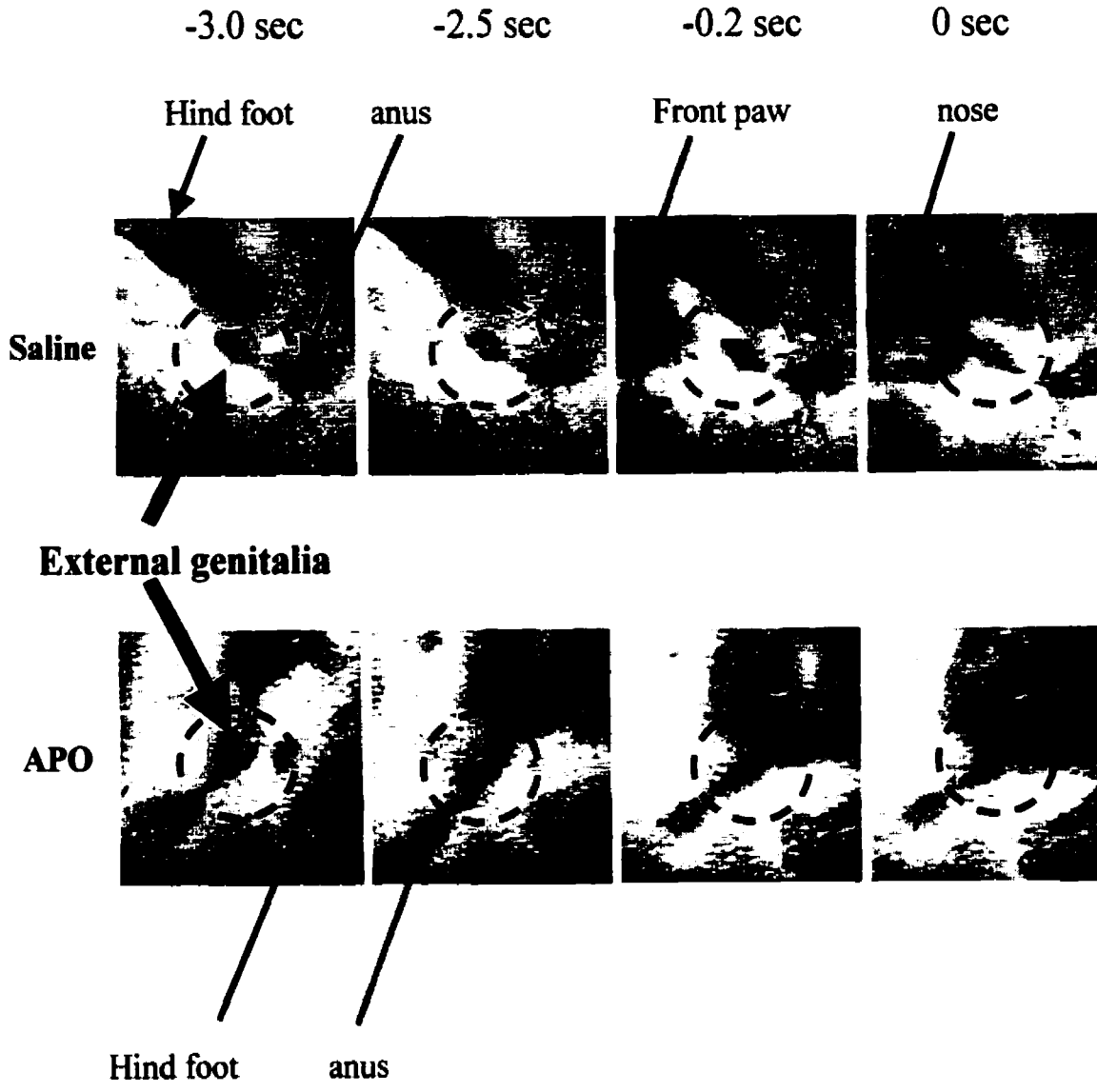

Figure 2-1: 3 second time course of genital response prior to the licking response in saline administered intact female rats (upper panel). There was no change in the appearance of the external genitalia (non-GVA).The lower panel demonstrates a 3 second time course of genital response prior to the licking response in APO 80μg/kg administered intact female rats. The external genitalia progressively became enlarged (GVA).

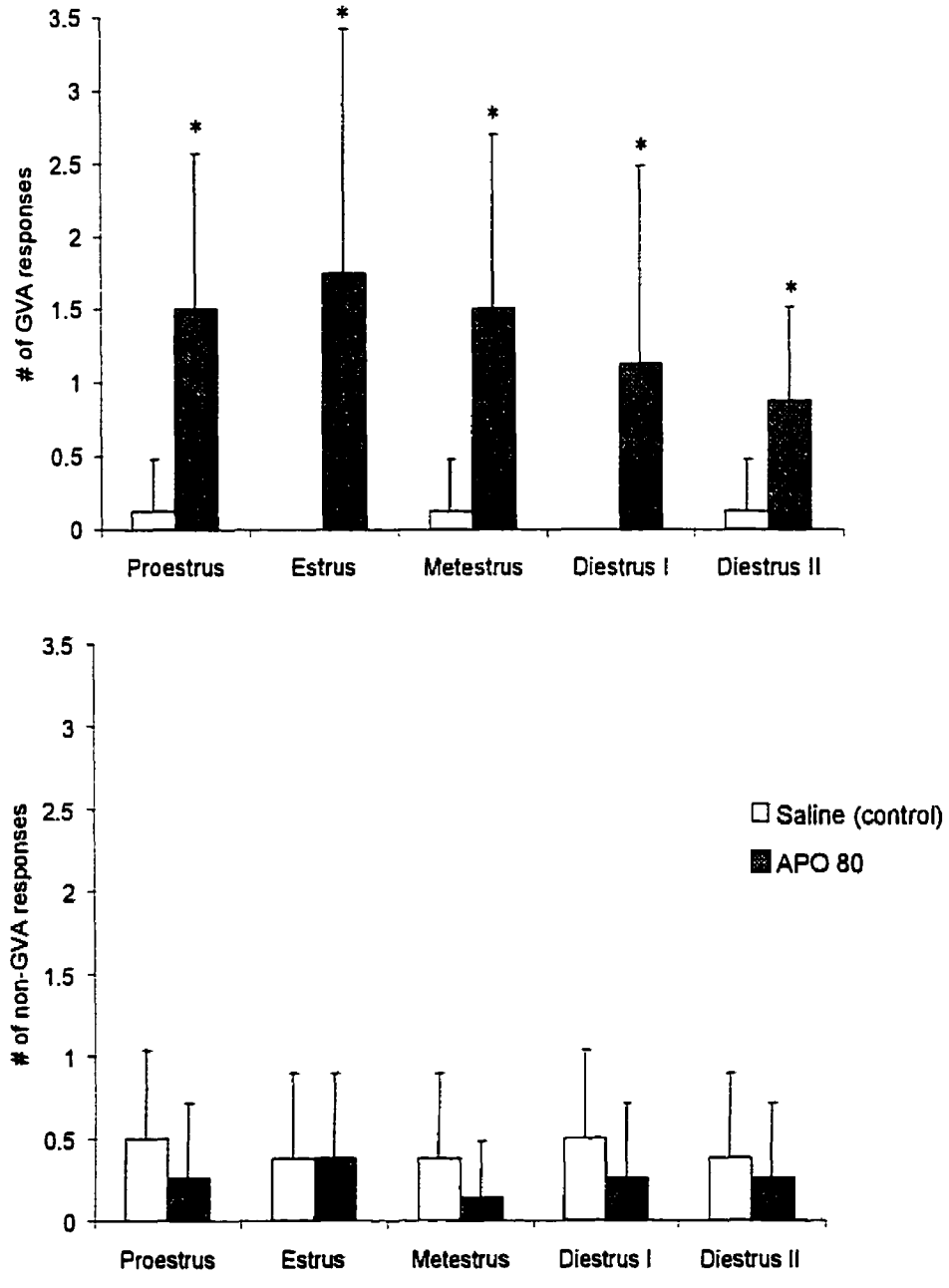

Figure 2-2: APO 80 μg/kg administration produced a significant increase in GVA responses in every stage of the estrous cycle over respective controls (upper panel). APO-induced GVA response peaked in the estrus stage of the estrous cycle. APO 80 μg/kg administration produced no significant differences in non-GVA responses (lower panel) in any stage of the estrous cycle over respective controls. Data expressed as mean ± std. deviation. * indicates significant difference from respective control $p<0.05$

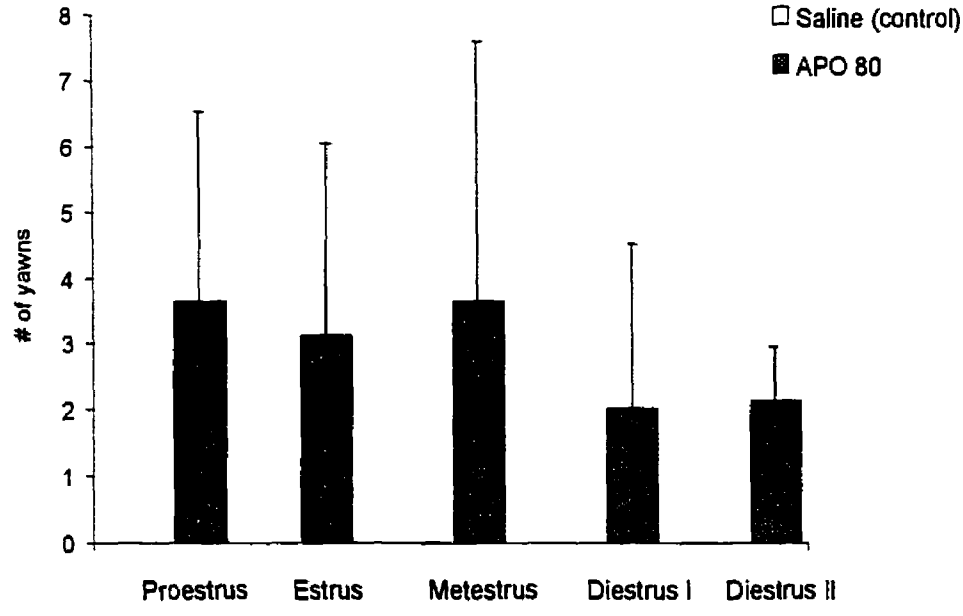

Figure 2-3: A yawning response was observed in all segments of the estrous cycle following APO 80μg/kg administration. There was no significant difference, among yawns, seen in any stage of the estrous cycle. There were no yawns observed in respective controls. Data expressed as mean ± std. deviation.

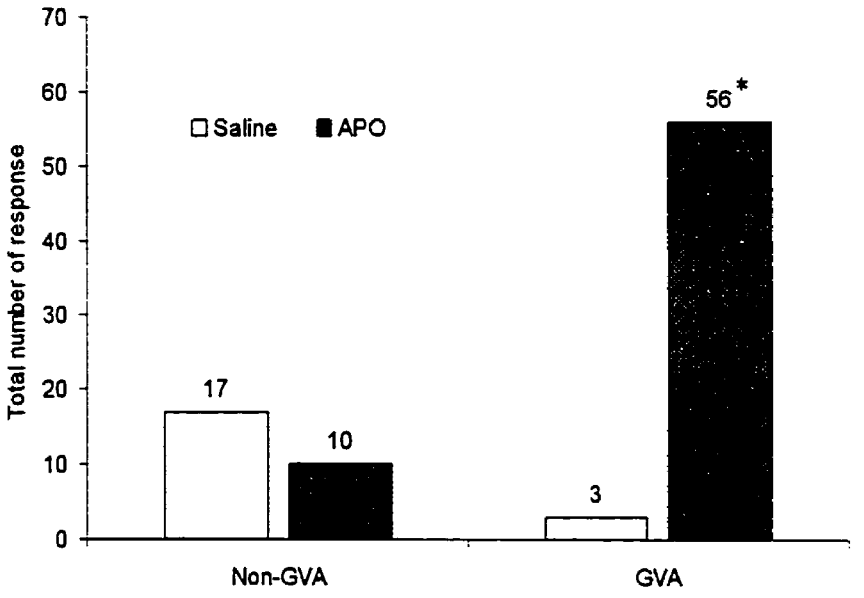

Figure 2-4: 3 GVA responses were observed throughout all stages of the estrous cycle after vehicle administration. Upon APO 80μg/kg administration the GVA response was increased to 56 over all stages of the estrous cycle. 17 non-GVA responses were observed throughout all stages of the estrous cycle after vehicle administration. Upon APO 80μg/kg administration 10 non-GVA responses were observed over all stages of the estrous cycle. There was no significant difference between APO tested and control animals. Data expressed as total number of responses over the estrous cycle. * indicates significant difference from respective control $p<0.05$

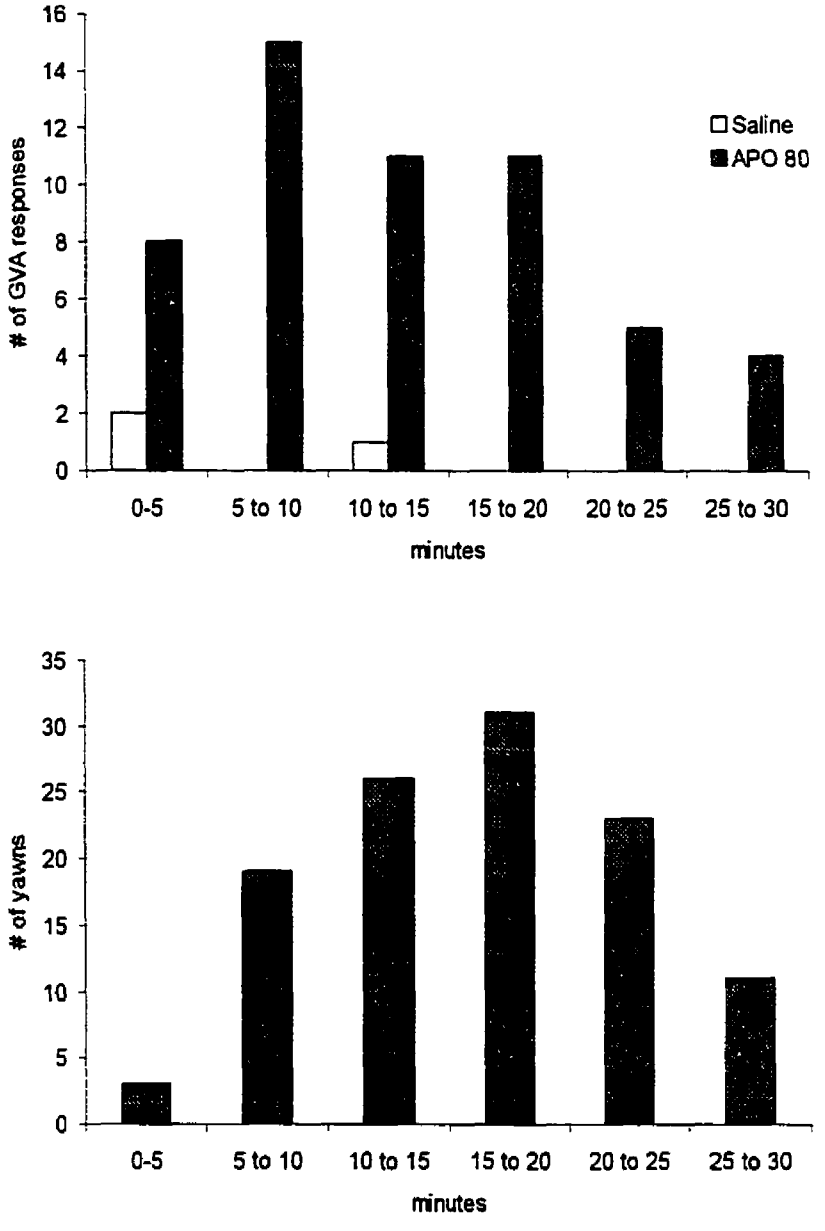

Figure 2-5: Upper panel shows time point distribution of genital vasocongestive arousal responses over the entire estrous cycle. This demonstrates that responses peaked at the 5-10 minute interval and was at its lowest at the 25-30 minute time interval. The data also shows that 80% of the GVA responses occurred within first 20 minute time period. The lower panel shows a time point distribution of yawns over the entire estrous cycle. The distribution was bell shaped with the peak response occurring at the 15-20 minute interval. Data expressed as total number of responses over the estrous cycle.

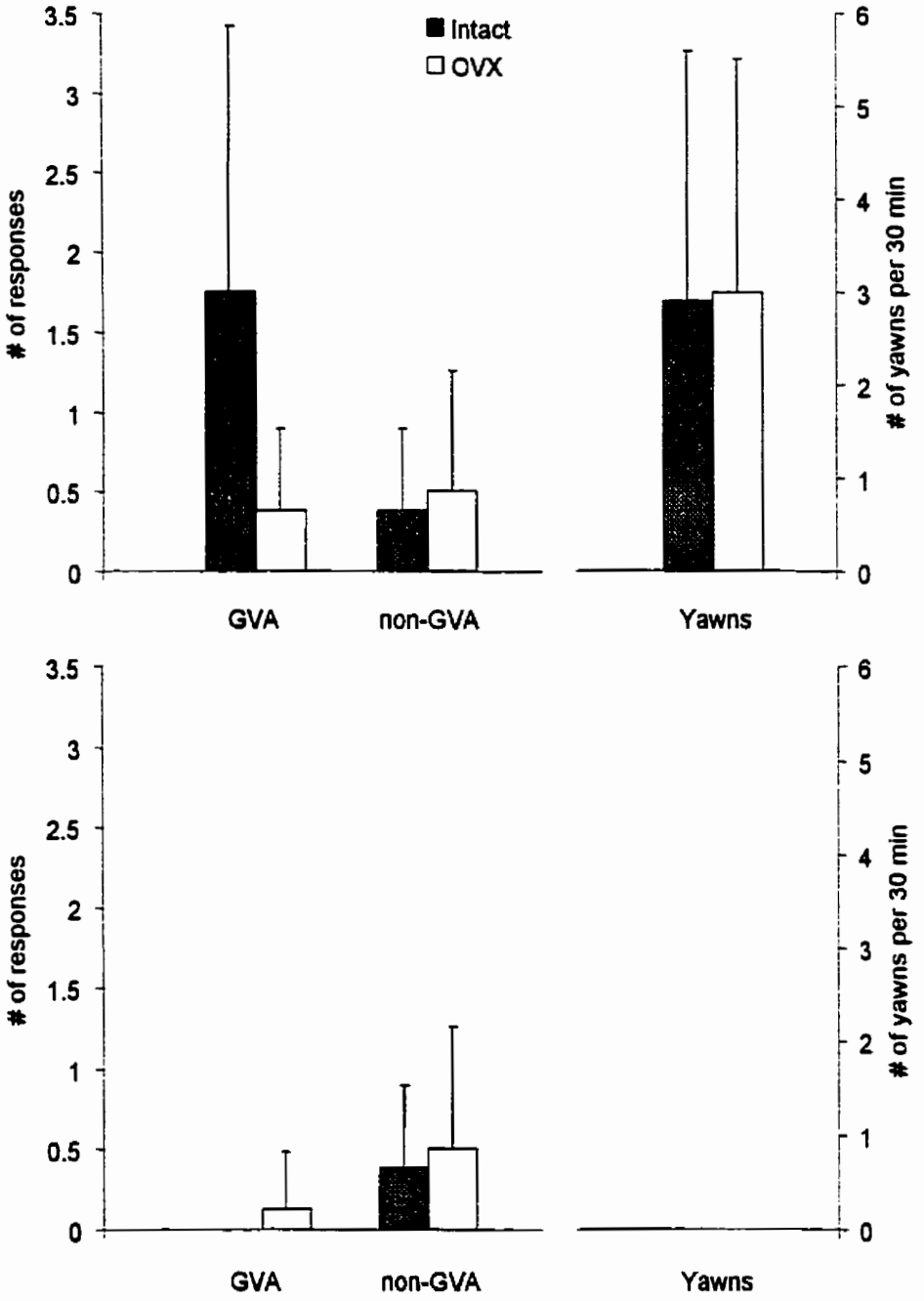

Figure 2-6: Upon OVX there was no significant change observed in either GVA or non-GVA responses to saline administration versus intact rats. No yawns were observed in either intact or OVX rats given saline (lower panel). Upon OVX there was a significant decrease in APO-induced GVA response versus intact animals. However there was no significant change in non-GVA responses versus intact animals after APO administration (upper panel). APO-induced yawns were observed in both intact and OVX animals. Data expressed as mean ± std. deviation. * indicates significant difference from respective OVX animal $p<0.05$

2.4 Discussion

The major finding in this study was that low doses of APO can centrally initiate a series of sexual responses in female rats characterized by both behavioral changes and genital vasocongestive arousal (GVA) events that are dependent on the hormonal milieu. Both the behavioral and vascular response (GVA) were found to be analogous to the APO-induced erectile response seen in the male rat [71] based on similar timing and frequency. Yawns were also observed after APO administration but were not causally or temporally associated with the behavioral or vascular events.

The patterned behavioral response seen in these female rats when administered APO is clearly consistent with the known central effects of APO. APO is a non-selective dopamine receptor agonist (D_1/D_2), when administered at low doses. It is widely acknowledged that APO actions include yawns and penile erections in male rats [74] [72] [90]. Previously, APO was shown to enhance lordosis behavior in female rats [69] [91] although genital responses have not been determined. As indicated, APO acts as a central initiator of penile erections and yawns by binding to the D_2 receptors of the paraventricularnucleus (PVN) of the hypothalamus. The PVN is known to be key in controlling the sexual response in male rats. In addition recent literature has suggested that the PVN plays a similar role in the female rat sexual response [9]. This is in keeping with our results where APO centrally initiated both behavioral and peripheral vascular (GVA) sexual responses via the D_2 receptors in the PVN.

In the present study, the peripheral manifestation of this central action was found to involve engorgement of the genitalia, a key component of the sexual vasocongestive arousal response [4] [3]. Our findings showed that the time course of the APO-induced GVA event was approximately 3 seconds. This time course was surprisingly similar to the time course of APO-induced erectile

responses seen in male rats. In both male and female rats treated with APO the rapidity of onset, the transient nature and the complex coordination of the response are all characteristics which confirm the response as centrally initiated and neurally mediated.

The APO-induced behavioral responses prior to genital engorgement in the both male and female rats are very similar. Both patterns consist of a series of changes including: a 'startle' response in which it appears that the rat's awareness of its surroundings is suddenly heightened, a movement involving the occurrence of concavity of the back leading to rearing and erect posture on hind legs. Following these events, the rapid descent of the head into the genital area occurs immediately prior to genital arousal (GVA in females, erection in males), and a final response that involves genital grooming.

The erectile response in male rats is known to be hormonally dependent. The erectile response can be eliminated with castration and restored with testosterone supplementation [49] [48]. Similarly, in the present investigation the APO-induced response in female rats appears to be hormonally regulated in frequency at different stages of the estrous cycle. The peak of the APO-induced GVA responses, similar to the natural sexual receptivity, was during proestrus, estrus, and declines to the nadir during late diestrus [92] [70]. This time course correlates with both estrogen and progesterone secretion. Estrogen secretion begins in late diestrus, peaks in proestrus and then serum levels rapidly decline in estrus [93]. While, progesterone peaks in proestrus and declines in estrus [94]. Thus, the heightened APO-induced GVA appears to be correlated, at least in part by a combination estrogen and progesterone secretion. Furthermore, the present data demonstrates unequivocally that the APO-induced GVA response is hormonally dependent in female rats as ovariectomy markedly diminished the response compared to the intact animals.

Based on the understanding that non-adrenergic non-cholinergic (NANC) nerves release nitric oxide (NO) and vasoactive intestinal peptide (VIP) as part of the neurogenic signaling in the female sexual arousal response, it is not surprising that NO and VIP activity are targets of hormonal regulation. Previous studies have demonstrated that the activity of nitric oxide synthase (NOS) in the rat genitalia peaks during estrus [95] indicating that this elevation is estrogen dependent [96]. Increased levels of NOS will subsequently facilitate increased blood flow in the genital area during times of sexual arousal. In addition, upon ovariectomy and the subsequent removal of the endogenous hormone estrogen, there is a substantial decrease in NOS activity in the rat vagina. [97] [62]. With regard to VIP, a previous study examined vaginal blood flow in response to this factor in postmenopausal women. It was demonstrated that VIP blood flow response was decreased in these postmenopausal women. However upon hormonal replacement therapy in these postmenopausal women the vasodilatory effect of VIP returned to levels similar to normal cycling women [98]. Taken together, it appears that sex hormones play an important role in the functional modulation of enzymes and receptors that may be used in facilitating the vasodilatory effects of local factors such as NO and VIP in sexual arousal. Further investigation with respect to the influence of endogenous hormones on neurally mediated factors involved in the sexual response are needed to better understand the impact of hormones on the regulation of blood flow to the genital area.

Previous assessments of sexual responses, particularly in female animals, have focused on behavioral responses as opposed to physiological mechanisms at the level of the genitalia. Recently the female New Zealand white rabbit was used as an animal model for vasculogenic female sexual dysfunction by observing the specific physiological impairment associated with vaginal vasocongestion and clitoral erectile insufficiency syndrome [3]. Upon assessment of the

literature it appears that the current study is the first conscious animal model that incorporates central initiation leading to a peripheral vascular response consistent with sexual vasocongestion.

In conclusion a rat model of female sexual response using apomorphine as a central initiator has been established. In human female sexual function one of the characteristics of female sexual arousal is increased blood flow to the genitals and the associated engorgement. The present studies have demonstrated that apomorphine can initiate genital vasocongestive arousal response in a manner convergent with the natural process. This model will likely have immediate utility in helping elucidate mechanisms of female sexual function and dysfunction.

Chapter 3: Dysfunction and recovery of genital vasocongestive arousal (GVA) response in the female rat: Role of testosterone and nitric oxide (NO)

3.1 Introduction

In Chapter 2 a conscious rat model of female sexual arousal induced by the dopaminergic agonist apomorphine was fully characterized [85]. The major event characterized in this model was genital vasocongestive arousal (GVA), involving engorgement of the external genitalia, a key component of the sexual arousal response [3]. In the female rat this event involves the same behavioral patterns and vascular events associated with the erectile response in male rats. Both the GVA and erectile response can be consistently reproduced with the administration of the central initiator apomorphine (APO). APO has been shown to elicit erections by acting on the D_2 receptors of the paraventricular nucleus (PVN) of the hypothalamus [72].

Endogenous sex hormones play a key role in regulating sexual function in both male and female rats. Removal of these hormones inhibits both sexual responses and behaviors, indicating that sex hormones are mediators at every level of the sexual pathway from higher brain regions to control of blood flow in the genitalia. Both GVA events and erectile responses have been shown to be hormonally dependent – in that ovariectomy (OVX) diminishes the GVA response in female rats, [85] while castration inhibits the erectile response in male rats [49]. Testosterone replacement restores the erectile response in castrated male rats [49]. Decreases in estrogen and progesterone after OVX have been associated with decreased sexual function, [53,46] however other studies have suggested that it is the decrease in free testosterone after OVX which is the major factor responsible for decreased sexual function [51]. Further, evidence for this has been demonstrated in post menopausal women who experience sexual dysfunction. Testosterone, along with other endogenous hormones, is produced in the ovary throughout life with their levels decreasing after menopause. Recently, testosterone treatment has been considered to be efficacious in treating women suffering from sexual dysfunction [99].

Nitric oxide (NO) is a non-adrenergic, non-cholinergic vasodilatory factor that is derived from the conversion of L-arginine to L-citrulline by nitric oxide synthase (NOS) [100]. It is widely accepted that NO plays a primary role in mediating penile erections. Studies that have acutely inhibited the production of NO via nitric oxide synthase (NOS) blockade have almost obliterated the erectile response [28]. Similar studies have not been performed in females, with respect to NOS blockade and genital vasocongestion. However, some studies have suggested that NO is a factor, both centrally and peripherally involved in regulating the female sexual response cycle. NO regulates neurotransmitter release in brain regions important in the mediation of the sexual response. NO neurons in the hypothalamus and medial preoptic area (MPOA) facilitate the release of dopamine that can initiate the sexual response [101]. Further, the vagina and clitoris have both been shown to be abundantly innervated by NO producing neurons [102]. During sexual stimulation at the level of the genitalia, NO has been suggested to be released from both neurons and endothelial cells based on the activation of guanylyl cyclase [38]. Guanylyl cyclase in turn converts guanosine triphosphate to cGMP that results in smooth muscle relaxation [37]. This suggests that NO is an important regulator in facilitating blood flow to the female genital area, especially the clitoris and vagina, during the female sexual arousal response.

Previous studies have demonstrated a dependence of NOS expression on endogenous hormones in both male and female rats [103]. For example, estrogen has been shown to increase NOS activity both centrally (hypothalamus) and in the periphery (vagina) [104] [46].

Using the APO treatment model, quantitative assessment of genital vasocongestive arousal (GVA) responses in female rats can be investigated under a variety of conditions. Therefore, in the present study, the impact of NO-mimetic treatment in a model of NO-deficiency, and hormonal supplementation in OVX rats was determined. Furthermore, given that the APO-

induced GVA response is reduced with OVX (removal of most endogenous sex hormones), and NOS activity has been shown to be dependent on estrogen, an additional goal of this study was to determine whether NO supplementation in OVX animals restores the APO-induced GVA response to levels comparable with intact animals.

3.2 Method and Materials

Animals

Adult female Wistar rats (225-250g) obtained from the Charles River Laboratories (Montreal, Canada) were used for the purpose of this study. Animals were housed in polypropylene shoebox cages in a climate-controlled room with a 12/12-hour light/dark cycle. Food (Purina rat chow ®) and water were provided *ad libitum* except during times of testing. The procedures carried out in this study were in accordance with the Canadian Council of Animal Care. Rats were handled for 5 consecutive days prior to experimentation to allow for acclimation to the procedure. Prior to experimentation, all rats showed a consistent 4-day estrous cycle consisting of normal periods of proestrus, estrus, metestrus and diestrus. Stages of the cycle were determined by analysis of epithelial cells taken from vaginal smears [87].

Experimental Procedure

Modifications to the APO (apomorphine, 80 ug/kg) protocol originally published by [74] were used for the purpose of this study [49]. In brief, APO (apomorphine hydrochloride - Sigma®) was prepared in solution with ascorbic acid (100 ug/ml). Rats were placed in hanging cages with bottoms constructed of Plexiglas®, in a dark, quiet room where they were allowed to acclimate for 10 minutes before the testing period. APO 80 ug/kg was injected subcutaneously (s.c.) and genital vasocongestive arousal (GVA) responses and associated yawning responses were observed and recorded during a 30 minute period via a video monitoring system in an adjacent room. A GVA response was counted when the rat demonstrated certain the following behavioral signs and genital responses: a 'startle' response in which it appears that the rat's awareness of its surroundings is suddenly heightened, a movement involving the occurrence of concavity of the

back leading to rearing and erect posture on hind legs. Following these events, there is a rapid descent of the head into the genital area, which becomes noticeably engorged and enlarged. Genital licking terminates the overall response [85]. A yawn was counted as an involuntary opening of the mouth with the appropriate respiratory movement.

Surgical procedure

Ovariectomy (OVX)

In brief, OVX was carried out using a similar procedure to Wayforth *et al* [89]. Animals were anaesthetized with isoflurane gas delivered via a Bain rebreathing system. A small midline dorsal skin incision was made approximately half way between the middle of the back and the base of the tail. Both ovaries were removed by ligating and severing the junction between the fallopian tube and uterine horn. Post-operative analgesia (Buprenex, dose of 0.05 mg/kg) and the antibiotic Tribrissen 24% (0.1ml/100 g of body weight) (Schering Canada inc.) were administered daily for 5 days. Animals were given at least 1-week recovery before the onset of experimentation.

Insertion of intra-venous (i.v.) catheter

In brief, rats were anaesthetized with Hypnorm®/Versed® (1:2:1 ratio of Hypnorm®:H_2O:Versed®: 3ml/kg i.p.). Catheters made from small bore Teflon® tubing (0.012-inch i.d., 30 gauge, Cole-Parmer, Laval, Quebec) and inserted into vinyl tubing (0.02-inch i.d., 23 gauge) were implanted into the abdominal aorta. All catheters were tunneled subcutaneously where they were exteriorized at the nape of the neck and sutured into place. The catheters were filled with heparinized saline (50 IU/ml) and sealed at the exposed end to prevent clotting at the inserted end.

Experiment A – APO-induced GVA response in ovariectomized (OVX) rats given testosterone supplementation.

OVX animals were used to ensure endogenous hormones were present at stable and minimal levels. The APO-induced GVA response was then re-assessed in the OVX rats 24, 36, 48 hrs following testosterone (testosterone propionate 480, 960 μg/kg, s.c., n=8) supplementation. The protocol was based on a previous time course assessment, showing the effect of testosterone peaking at 36 hrs prior to APO administration [49]. APO-induced GVA responses were determined as described above.

Experiment B – Induction of nitric oxide (NO) deficiency and subsequent NO-mimetic supplementation on APO-induced GVA response in intact rats.

The nitric oxide synthase (NOS) inhibitor, L-NAME (N-Nitro-L-Arginine methyl ester (sigma®), 30 mg/kg), was administered to each of 8 intact rats via the i.v. line 30 min prior to APO testing. APO-induced GVA responses were determined as described above.

The effect of a low dose infusion of a NO-mimetic (sodium nitroprusside (SNP) (Sigma®) 10 ug/kg/min), after NOS blockade on APO-induced GVA responses was also assessed. In preliminary studies this dose of SNP was shown to restore the mean arterial pressure to normal levels in L-NAME treated animals and was also shown to decrease blood pressure by only 5 mmHg in untreated animals. The NO-mimetic was given, via continuous i.v. infusion using a infusion pump (Fisher Scientific®), 15-20 minutes after the NOS inhibitor was administered. NO-mimetic infusion continued throughout the 30 min APO testing observational period. The

APO-induced GVA response was determined using methods described above. The amount of SNP infused was determined previously.

Experiment C – NO-mimetic supplementation on APO-induced GVA responses in OVX rats.

The effect of NO-mimetic (sodium nitroprusside (SNP) (Sigma®) 10 ug/kg/min), administration on APO-induced GVA responses in each of 8 OVX rats was observed. The NO-mimetic was given, via continuous i.v. infusion using an infusion pump (Fisher Scientific®) just prior to APO testing. NO-mimetic infusion continued throughout the 30 minute APO testing observational period.

Data analysis

GVA responses

Statistical analysis among treatment groups was performed using Kruskal-Wallis one way ANOVA on ranks with Dunn's post hoc. ($p<0.05$)

Yawning response

Statistical analysis among treatment groups was performed using one way ANOVA ($p<0.05$)

3.3 Results

Genital vasocongestive arousal

As found in chapter 2, APO (apomorphine, 80 ug/kg) causes a genital vasocongestive arousal response in female rats [85]. This response is analogous to the APO-erectile response in male rats [74] with respect to timing, frequency and rapidity of onset. As described in Chapter 2, the GVA response, similar to the erectile response, is preceded by a patterned behavioral response involving: a 'startle' response in which the rat's awareness of its surroundings is suddenly heightened. The 'startle' response is followed by a concavity of the back, rearing, in which the rat demonstrates an erect posture by standing on its hind legs and vasocongestion of the external genitalia. Consequently, the rat rapidly descends head first into the engorged genital area (GVA), and proceeds to complete this behavioral response with genital grooming.

APO-induced GVA response in ovariectomized (OVX) rats given testosterone supplementation.

APO -induced GVA responses were determined in 8 OVX Wistar rats and the number of responses for each rat in the subsequent 30 minutes was counted. There was almost a 75% reduction in the number of APO-induced GVA responses observed in OVX versus intact rats (figure 3-1). Assessment of the dose-response (480, 960 ug/kg) and time course (24, 36, 48 hrs) effects of testosterone on the GVA responses (table 3-1) revealed that the peak effect occurred at a dose of 960 ug/kg of testosterone administered 36 hrs prior to APO testing. Upon testosterone replacement with a dose of 960 ug/kg the APO-induced GVA responses were restored to levels not significantly different from those seen in intact rats (figure 3-1). The APO-induced yawning

response did not significantly change in either the OVX or OVX-testosterone combination treatment groups compared to intact animals (figure 3-1).

APO-induced GVA response in intact rats: Induction of nitric oxide (NO) deficiency and effects of subsequent NO-mimetic supplementation

APO-induced GVA responses were determined in 8 intact female Wistar rats and the number of responses for each rat in the subsequent 30 minutes was counted. NO deficiency induced by administration of the nitric oxide synthase (NOS) antagonist L-NAME (30 mg/kg, i.v.) significantly reduced the average number of APO-induced GVA responses in the intact animals by almost 80% (figure 3-2). Subsequent NO-mimetic supplementation (SNP, 10 ug/kg/min) in these NO deficient animals almost completely restored the APO-induced GVA response to control levels (figure 3-2). The NO deficiency appeared to reduce the number of yawns seen compared to control although this difference was not found to be statistically significant (figure 3-2).

NO-mimetic supplementation in OVX rats

Baseline APO-induced GVA responses were determined in 8 intact female Wistar rats prior to OVX. Infusion of a NO-mimetic (SNP, 10 ug/kg/min) at a dose which recovered GVA responses in NO-deficient animals did not have any impact on the APO-induced GVA response in the OVX animals (figure 3-3). Further, the APO-induced yawning responses were also not significantly altered in either the OVX or OVX+SNP treated groups compared intact animals (figure 3-3).

		Time course of APO administration after testosterone pretreatment (hrs) n=8		
		24	**36**	**48**
Testosterone (480 µg/kg)	*GVA*	0 ± 0	0.25 ± 0.46	0.25 ± 0.46
	Yawns	2.0 ± 2.20	3.0 ± 4.69	2.0 ± 2.82
Testosterone (960 µg/kg)	*GVA*	0.5 ± 0.58	**1.0 ± 1.77**	0.25 ± 0.46
	Yawns	4.0 ± 5.22	4.13 ± 4.19	1.88 ± 2.30

Table 3-1: Assessment of the dose-response and time course of the GVA and yawning response to APO (80 ug/kg) following treatment with testosterone in OVX rats. The maximal APO-induced GVA response occurred after 36 hrs. pretreatment, using testosterone 960 ug/kg.

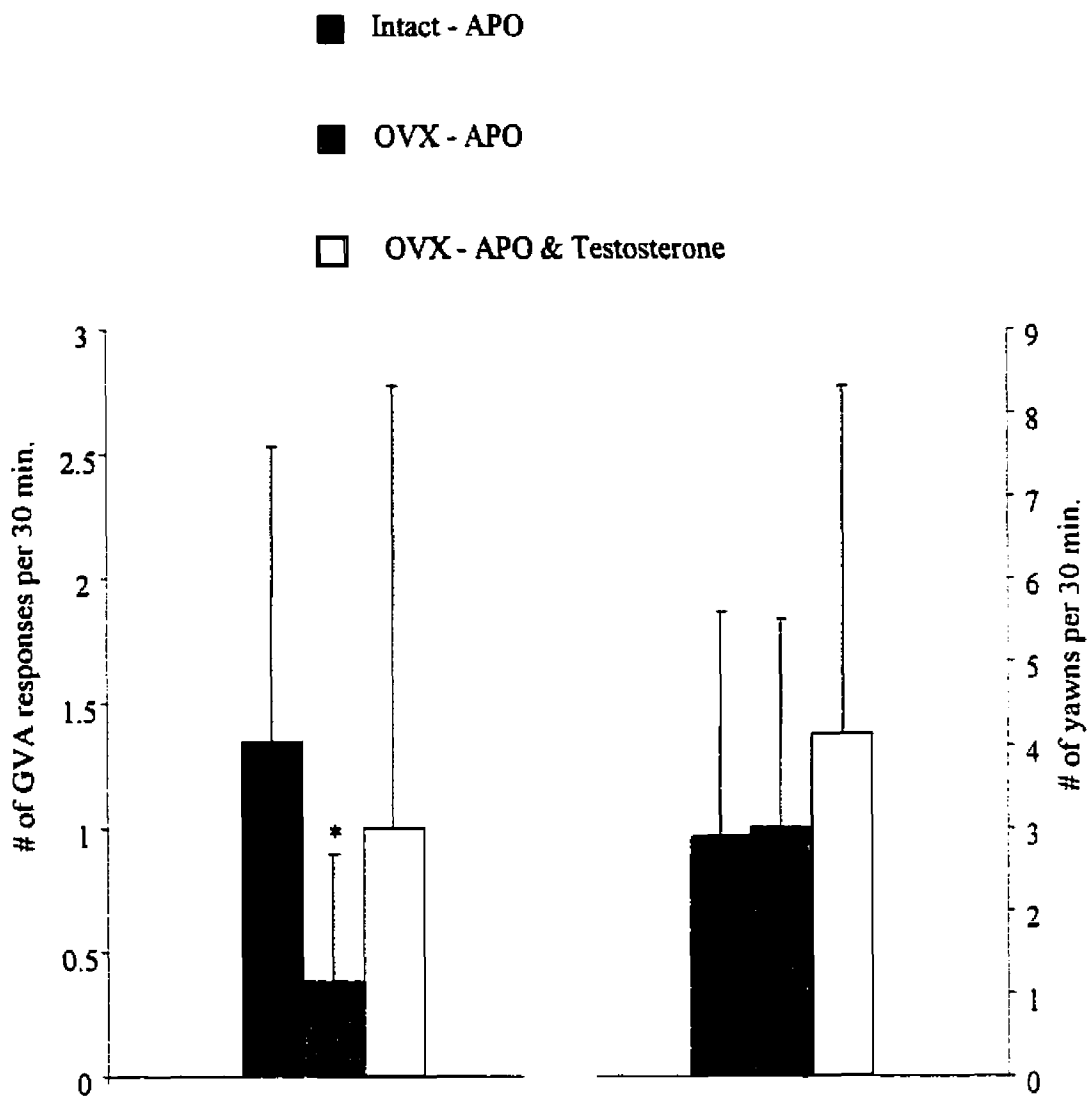

Figure 3-1: OVX resulted in a significant decrease in the APO-induced GVA response in the female rat. Administration of testosterone (960 ug/kg) 36 hrs prior to APO (80ug/kg) restored the GVA response to levels comparable with those of intact animals. There was no significant change in the yawning response in any of the treatment groups. Data expressed as mean ± std. deviation. * indicates significant difference from intact animal $p<0.05$

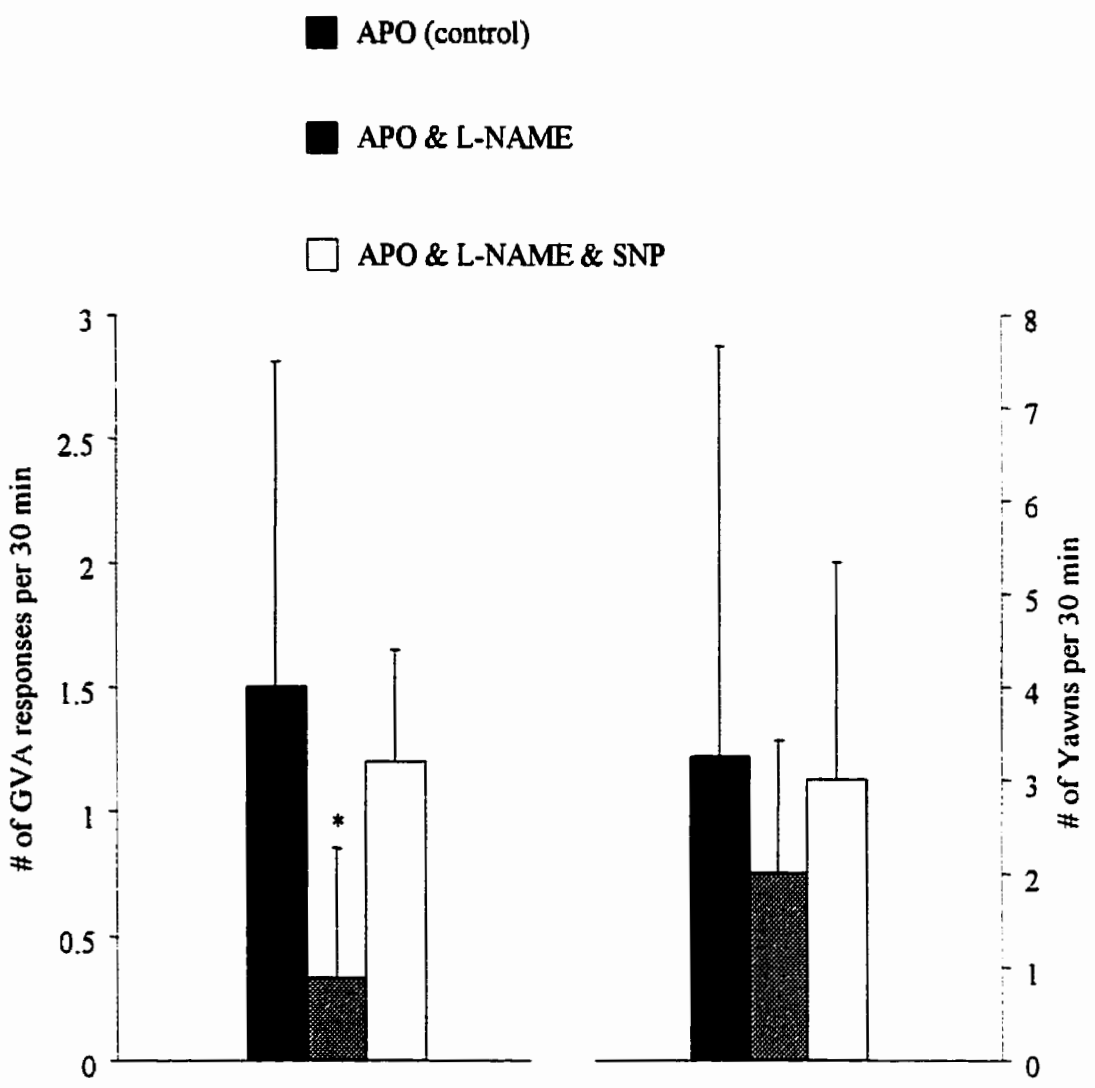

Figure 3-2: NOS blockade (L-NAME 30 mg/kg) resulted in a significant decrease in the APO-induced GVA response in the female rat. NO supplementation (SNP 10 ug/kg/min, iv) restored the GVA response to levels comparable with those of control (APO 80 ug/kg) animals. There was no significant change in the yawning response in any of the treatment groups. Data expressed as mean ± std. deviation. * indicates significant difference from control animal $p<0.05$

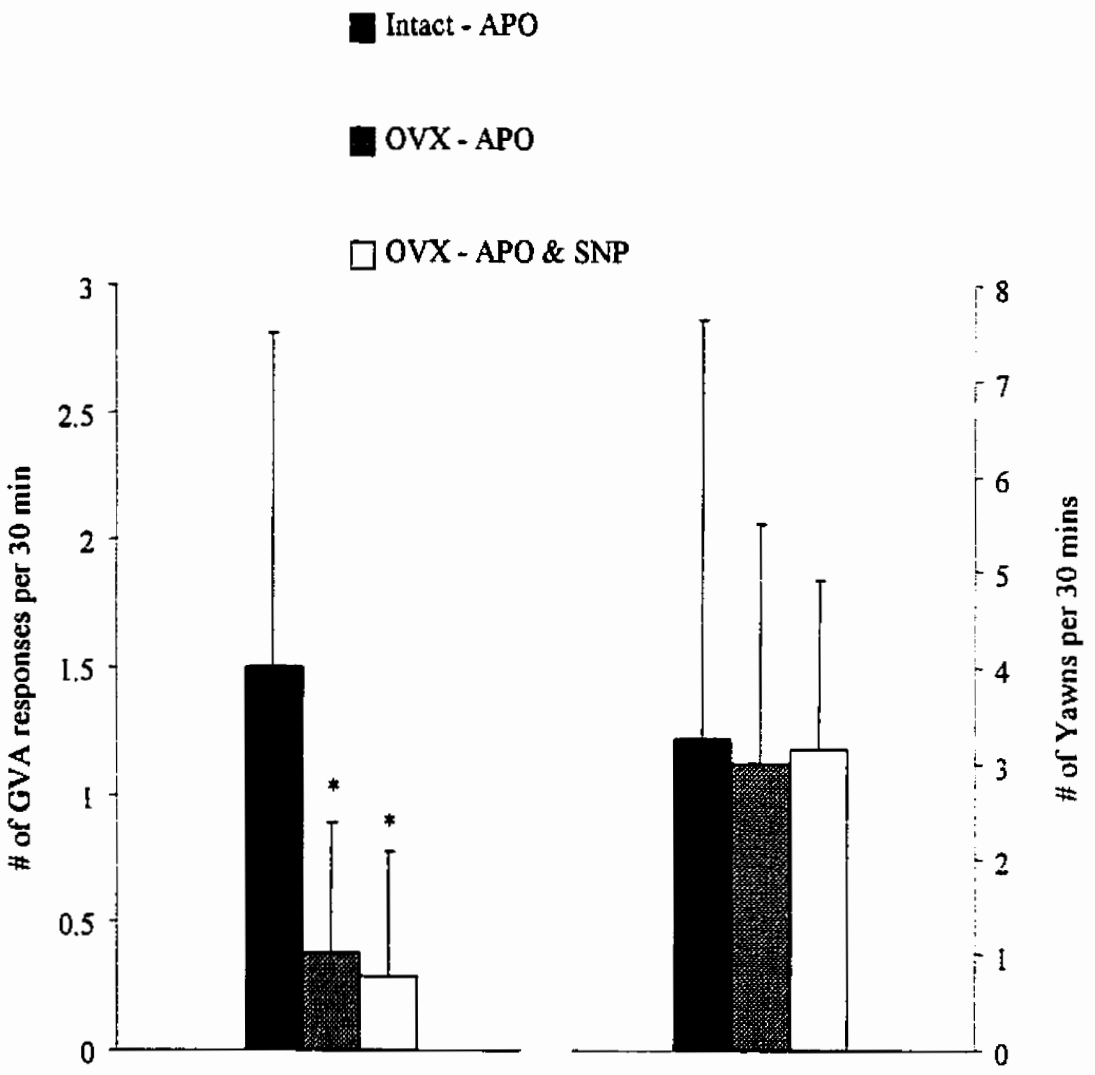

Figure 3-3: OVX resulted in a significant decrease in the APO-induced GVA response in the female rat. NO supplementation (SNP 10 ug/kg/min, iv) did not restore the GVA response to levels comparable with those of intact (APO 80 ug/kg) animals. There was no significant change in the yawning response in any of the treatment groups. Data expressed as mean ± std. deviation. * indicates significant difference from intact animal $p<0.05$

3.4 Discussion

The major findings of this study demonstrate the importance of endogenous hormones and nitric oxide (NO) in the regulation of the apomorphine (APO)-induced sexual response in the female. Specifically, the APO-induced sexual response in female rats was significantly reduced following ovariectomy (OVX) and by inducing a condition of NO-deficiency. Restoration of the APO-induced sexual response was achieved in OVX and NO-deficient animals upon exogenous testosterone and NO-mimetic supplementation, respectively. However, NO-mimetic administration did not improve the responses in OVX rats.

In OVX rats both the behavioral and vascular response associated with the APO-induced genital vasocongestive arousal (GVA) were significantly diminished, consistent with the previous findings [85]. Previously, the dependence of erectile functioning on testosterone in male animals and man has been well established. Castration in male rats markedly diminishes the APO-induced erectile response, while testosterone administration restores it [49].

The presence and location of androgen receptors in the central and peripheral nervous system indicate that testosterone plays a role in mediating a penile erection. The deprivation of androgens (after castration) can influence synaptic connections in the spinal cord by decreasing the somal size and dendrite length of spinal motorneurons that innervate the bulbo-cavernosal muscle of the penis. However these decreases can be reversed with testosterone supplementation [105] [106].

Estrogen and progesterone are involved in controlling a number of central and peripheral mechanisms associated with sexual behavior in female rats. CNS effects of estrogen and progesterone have been shown to include regulation of brain areas involved in sexual behavior such as the hypothalamus and the medial preoptic area (MPOA) [107] [108] [109]. A potential

mechanism by which estrogen and progesterone may facilitate sexual behavior centrally is by increasing the release of neurotransmitters, for example NO, dopamine and oxytocin [110] [111] [112]. These neurotransmitters are widely accepted as important mediators in sexual behavior [110] [111] [112]. This would indicate that estrogen and progesterone are essential factors in many areas of the female sexual response pathway. Administration of a combination of estrogen and progesterone to OVX animals has been shown to facilitate sexual behavior [107] [81]. However the temporal relationship between administration of each individual hormone is critical for induction of this sexual behavior. Studies have shown inhibitory actions of progesterone and estrogen on OVX females depending on the time course chosen for individual hormone administration with respect to the other [113]. However, sole administration of testosterone has been shown to restore female sexual behavior in OVX animals [114] [115]. In the present study exogenous testosterone (testosterone propionate, 960 ug/kg) supplementation was shown to restore both the behavioral and genital response associated with the (APO)-induced GVA response in ovariectomized (OVX) rats. The restoration of the response occurs 36 hours after hormonal administration. This time course suggests that testosterone does not act directly to increase blood flow to the genitalia but causes some induction of change in the sexual response pathway that can facilitate the GVA response.

The APO-induced erectile response and the APO-induced GVA response in female rats appear to be analogous sexual responses [85]. Both APO-induced responses are centrally initiated via dopamine type 2 (D_2) receptors in the paraventricular nucleus (PVN) of the hypothalamus [72] and are mediated through similar pathways in the peripheral nervous system [75] [78]. In addition both responses demonstrate similar behavioral and genital vascular vasocongestive events [85]. The present study demonstrates that testosterone can restore the APO-induced GVA response in

OVX female rats in a manner similar to the recovery of erectile response in the male rats. Taking previous studies and results found here it appears that testosterone modulates central mechanisms associated with the sexual response pathway.

Another major finding of this study showed the importance of NO in the mediation of the APO-induced GVA response in female rats. Similar to the erectile response, NO has been implicated as the major factor in regulating blood flow associated with increased female sexual arousal [38] [3]. By blocking the production of NO using a NOS inhibitor we were able to markedly diminish the GVA responses, an effect which was reversed by subsequent administration of a NO-mimetic.

NOS activity appears to be regulated by endogenous sex hormones in both males and females. For example, Upon OVX and subsequent removal of estrogen, NOS activity both centrally and in the periphery, has shown to be significantly decreased [46] [116]. Similarly, a downregulation of nitric oxide synthase (NOS) fibres occurs in the corpus cavernosum upon testosterone removal [103]. As indicated earlier, NO which is produced by NOS has been identified as a principle mediator in regulation of penile erections and blood flow to the female genitalia.

Therefore in the present study given that NO release is affected in OVX rats, the final step in this study was to investigate if NO supplementation could restore the APO-induced GVA response in OVX female rats. Upon NO supplementation to the OVX rats there was no increase in the APO-induced GVA responses. This inability of NO-mimetic supplementation suggests that the endogenous hormones, such as estrogen, may mediate a number of other factors that are necessary to accomplish this GVA event.

In conclusion this study demonstrates the importance of endogenous hormones and nitric oxide in regulating the sexual response of female rats. Specifically the ability of testosterone to recover

the sexual response in OVX female rats. In addition the NO was recognized as an important factor in mediating the genital vasocongestive response in female rats. The findings in this study can lead to further understanding of factors (hormonal, endothelial, neurogenic) that are responsible for the decline in sexual function in post-menopausal individuals and possible therapeutics in treating this disorder.

Chapter 4: General Discussion

Although lagging behind the research of male erectile dysfunction, the investigation of the physiology and pathophysiology of female sexual dysfunction is undergoing rapid expansion. Animal models provide a means by which a better understanding of the etiology and physiology/pathophysiology of a function/dysfunction can be achieved. In this thesis a rat model of female sexual arousal was established, based on the APO-induced erectile bioassay developed by Heaton *et al* [74]. Using APO (apomorphine, 80 ug/kg), we were able to initiate a centrally mediated sexual response in the female rat involving a patterned behavioral response and an associated physiological genital response. The physiological genital response appears to be characteristic of a genital vasocongestive arousal (GVA) event similar to the erectile response seen in the male rat with respect to rapidity of onset, timing and frequency. In addition, similar to the male erectile response, important mediators such as sex hormones and nitric oxide (NO) were identified as important factors in the sexual response pathway.

The major findings in this thesis:

a) Treatment with APO induced behavioral responses prior to genital engorgement in the both male and female rats that were very similar. Both patterns consisted of a series of changes including: a 'startle' response in which it appears that the rat's awareness of its surroundings is suddenly heightened, a movement involving the occurrence of concavity of the back leading to rearing and erect posture on hind legs. Following these events, the rapid descent of the head into the genital area occurs immediately prior to genital arousal (GVA in females, erection in males), and a final response that involves genital grooming.

b) The dependence of the APO-induced GVA response upon hormonal conditions was demonstrated in this study. The APO-induced GVA response was found to fluctuate with the natural hormonal cycle of the female rat – peaking during periods of time when estrogen and

progesterone exert their effects and declines to the nadir during times of decreased hormonal influence. The present data also demonstrated unequivocally the degree of hormonal dependence of the APO-induced GVA response in female rats, since ovariectomy markedly diminished the response compared to the intact animals. Testosterone supplementation 36 hours prior to APO testing restored the APO-induced GVA response to intact levels.

c) The importance of NO in regulating blood flow to the genitalia was shown in this study. Upon the removal of endogenous NO with a nitric oxide synthase (NOS) inhibitor we were able to significantly reduce the APO-induced GVA response. The genital responses were restored with concomitant NO-mimetic supplementation.

d) The interplay of these two factors – removal of hormones and NO by investigating the ability of NO to restore the APO-induced sexual response in OVX rats was assessed. Previous studies have shown the dependence of NOS expression on endogenous hormones [46]. However, the results demonstrated an inability of NO to restore the APO-induced sexual response in OVX rats. This suggests that downregulation of NOS expression is only one of the possible hormone-related factors involved in mediation of the sexual response in the female rat.

4.1 APO-induced GVA model – Further understanding the female sexual response pathway

The animal model developed in this study will provide a means by which the sexual arousal response can be further understood. Since the APO-induced GVA response is a peripherally

manifested event (i.e. patterned behavioral response and engorgement of the genitalia) which is centrally initiated, it will allow for investigation into the entire sexual arousal response pathway.

The erection being the physiological endpoint in the male has made it possible for the development of numerous animal models to explore the male sexual response pathways. Most of the female animal models in the past have been behavioral in nature. These behavioral models lacked a physiologically relevant end point in the completion of the sexual response that was similar to the erection in the male. Until the present studies there has been no conscious intact animal model that could demonstrate the increased blood flow and subsequent genital vasocongestion that occurs as a result of sexual arousal stimulus. The lack of availability of this physiological endpoint made it very difficult to explore the pathways involved in the female sexual response. The APO-induced GVA model, in this study, is the first conscious female animal model where there is an observable genital response associated with a sexual arousal stimulus.

As a result of the present research, it can be concluded that the APO-induced GVA response in females is analogous to the APO-induced erectile response seen in male rats. The PVN has been shown to be an important central site in the mediation of male erection and it has been suggested that it plays a similar role in female genital blood flow responses [117] [72] [9]. Thus, the APO-induced GVA response in the female rats can be used to demonstrate the specific role of the PVN in the female sexual response. Melis *et al* determined that the PVN was the site of action of APO by microinjecting APO into different brain regions of the male rat and observing the subsequent APO-induced penile erections [72]. Application of APO directly onto the PVN produced the greatest number of penile erections as compared to any other region. Similar

studies could be performed in female rats to determine/confirm the specific site of the APO-induced GVA responses.

Using the GVA response in this model, one can address the role that the peripheral nervous system (PNS) plays in mediating this response. Previously, studies by Paick *et al* used techniques such as nerve stimulation and nerve ablation to investigate the role that peripheral nerves play in regulating the APO-induced erectile response [75]. Through these types of studies a better understanding of the PNS involvement in the regulation of the male sexual response has been achieved. Using the GVA response as the physiological end point in the sexual response pathway, similar approaches can now be used in addressing the role that the peripheral nervous system (PNS) in mediating the female sexual response.

The model developed in this study has already shown the importance of hormonal factors and vasodilatory NANC factors (NO) in the mediation and regulation of the female sexual arousal response by demonstrating the impact that these factors have on the GVA response. There are many other cholinergic, adrenergic and NANC factors that have been implicated in genital blood flow during sexual arousal that could be similarly assessed. By the systemic or local administration of cholinergic/adrenergic agonists and antagonists, enzyme inhibitors and NANC mimetics the APO-induced GVA model can be used to address the roles and the importance of these factors in regulating blood flow in the genitalia during sexual arousal.

4.2 APO-induced GVA model – clinical relevance

The APO-induced model of GVA appears to be a good animal model in which female sexual arousal disorder (FSAD) can be investigated. FSAD involves an inability to respond to or maintain an adequate lubricative or swelling (vasocongestive) response during sexual stimulation

[1]. This disorder has been linked to the impairment of physiological processes, such as inadequate blood flow to the genitalia [7]. The major physiological event in the APO-induced GVA event is centrally initiated genital engorgement. Therefore, this model provides an excellent opportunity to parallel human sexual arousal experimentally and ultimately allow for the development of APO as a possible therapeutic for sexual arousal disorder in human females.

There are many disease processes associated with FSAD. Hypertension, diabetes and hypercholesterolemia have all been implicated as disorders that can impair blood flow to the genital area [30] [79]. The animal model developed in this study can, through the GVA responses, quantitatively assess and characterize the impact that the pathophysiology of the disease processes have on sexual arousal.

In conclusion, a rat model of FSAD has been developed and characterized in this study. Specifically addressing the roles endogenous hormones and NO play in mediating the female rat sexual response pathway. This model should prove helpful in elucidating the etiology, pathophysiology and possible therapeutics of female sexual function / dysfunction.

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