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Research Article

Courses of Arginine-Vasopressin in the Systemic and Cavernous Blood through Different Stages of Sexual Arousal in Healthy Males and Patients with Erectile Dysfunction

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To investigate the role of the peptide arginine-vasopressin (AVP) in controlling the function of penile erectile tissue, we determined the course of AVP through different stages of sexual arousal in both the systemic and cavernous blood of healthy males and patients presenting with ED. Twenty-five healthy males and 45 patients with ED were exposed to erotic stimulation to induce sexual arousal. Blood was withdrawn from the corpus cavernosum and a cubital vein during penile flaccidity, tumescence, rigid erection (attained only by the healthy individuals), and detumescence. AVP (ng/l plasma) was determined by means of a radioimmunoassay. Effects of AVP (0.1 to 100 nM) on isolated human CC were examined using a tissue bath system. AVP elicited contraction of isolated CC. In the healthy subjects, a decline in AVP levels (5.4 to 3 ng/l) was seen in the systemic blood when the flaccid penis became rigid. In the cavernous blood, no alterations were registered. In the group of ED patients, AVP in the systemic circulation did not display a transient decline. The drop in systemic AVP in healthy males during sexual stimulation might be a prerequisite to enable penile erection.

1. Introduction

The human sexual response cycle in males has been described as being divided into consecutive distinct phases that do normally involve desire followed by arousal and orgasm, including ejaculation. When sexual stimuli reach the central nervous system, pathways are activated to communicate signals from central centers to the genital tract through the spinal cord and the autonomic nervous system in order to elicit penile erection. Various neurotransmitters and neuropeptides have been identified to being involved in the control of the different stages of the sexual response at both the central and peripheral sites of action. These signaling pathways include oxytocin, adrenocorticotropin/the adrenocorticotropin-releasing hormone, the melanocyte-stimulating hormone, and opioidergic peptides [1–4]. Interactions with other neurotransmitters, such as nitric oxide (NO), gamma-aminobutyric acid (GABA), and dopamine, also occur in response to sexual stimulation, thereby enabling the entire course of different events composing the response cycle [5–7]. There is evidence that other peptides, namely, the gonadotropin-releasing hormone, corticotropin-releasing factor, vasoactive intestinal peptide (VIP), neuropeptide Y, substance P, and arginine-vasopressin (AVP), also play a role in the control of sexual function. AVP, also known as antidiuretic hormone (ADH), is synthesized in the hypothalamus and deposited in the pituitary gland. Following a respective stimulus, the peptide is

released into the blood stream. The half-life time of the circulating peptide varies between 10–35 minutes; it is metabolized in the liver and kidney by vasopressinase enzymes. Some of the main functions of vasopressin are the control of blood pressure and the constriction of arterioles, leading to an increase in the resistance of the vascular bed in the distal renal tubules and collecting ducts, thereby inhibiting diuresis and promoting the reabsorption of free water into the circulation [8, 9]. AVP is also widely distributed in sympathetic nerve fibers innervating the penile tissue and has been supposed to counteract the action of other neuropeptides, such as substance P and VIP, known to facilitate sexual activity [10–13]. This is, in part, in accordance with reports that injection of AVP into the ventricles of the lateral brain of male rats did not promote sexual behavior [14]. AVP can potentially contract isolated human penile blood vessels and the corpus spongiosum, these effects are almost completely inhibited by AVP antagonists [15]. By using radioimmunometric methods, AVP has been detected in human cavernous tissue [16]; however, a local uptake or synaptic release of AVP in the corpus cavernosum with the induction of penile rigidity (in response to visual sexual stimulation) or when the erected penis returns to the flaccid state, respectively, could not be proven by the findings from a study conducted in healthy male subjects by Becker *et al.* [17]. Given the incomplete understanding of the significance of AVP in the mechanisms controlling corpus cavernosum vascular and non-vascular smooth muscle tone and its potential role as one of multiple factors contributing to the etiology of male erectile dysfunction (ED), the present study is aimed at determining the courses of AVP through the stages of sexual arousal (as exemplified by different conditions of the penile erectile tissue) in the systemic and cavernous blood of healthy males and patients presenting with ED.

2. Material and Methods

2.1. Tissue Bath Studies (Contraction-Response Studies). Penile erectile tissue (corpus cavernosum) was obtained from 5 individuals who had been subjected to gender reassignment surgery (male-to-female). Tissue bath experiments were conducted according to standard procedures, as has been described earlier [18, 19]. A passive tension of 0.5 gr (5 mN) was applied to the strip preparations, followed by a resting period of 60 min. The alpha-adrenergic agonist norepinephrine (NE) or AVP was then applied in a cumulative manner to the bath chambers (0.1 nM–100 nM), and contraction responses were recorded using a MacLab™ system (ADI Pty. Ltd., Castle Hill, NSW, Australia).

2.2. Blood Withdrawal. Approval of the study was granted by the local ethics committee. Twenty-five (25) healthy males (mean age: 26 years) with normal erectile function and 45 subjects with erectile dysfunction (mean age: 52 years) were empaneled into the protocol. ED was assessed by means of a shortened questionnaire based on questions 1, 3, 4, and 5 of the International Index of Erectile Function. Frequent concomitant diseases to the ED were high blood pressure ($n = 17$), cardiac insufficiency ($n = 10$), atherosclerosis ($n = 8$), diabetes ($n = 9$), respiratory dysfunction ($n = 6$), and/or alcohol abuse ($n = 4$). Psychogenic patients ($n = 17$) were characterized by abnormal findings in the psychosexual evaluation but showed no signs of neurological disease and no pathological duplex sonography after injection of 5–20 μg prostaglandin E1. None of the volunteers had known pituitary diseases or an immanent condition of hyposexuality. A 20 G cannula (Vasofix™ Braunüle) was inserted into a cubital vein (CV) and a 19 G needle into the corpus cavernosum (CC). Blood samples were drawn from the cubital vein and the CC in the absence and during sexual arousal—as exemplified by the penile conditions flaccidity, tumescence, rigidity (attained only by the healthy individuals), and detumescence—and immediately stored on ice. Tumescence and rigidity of the penis were induced by presenting the participants sexually explicit audiovisual material combined with stimulation of the glans penis. The different phases of erection were visually defined by the investigator who was present in the room during the entire session. To avoid rapid degradation of peptides, the collection syringes (9 ml) contained the kallikrein protease inhibitor aprotinin (500 IU/ml) (Trasylol™, Bayer AG, Leverkusen, Germany). In order to separate the plasma, blood samples were centrifuged for 10 min (at +4°C) at 3000 rpm.

2.3. Determination of AVP. The extraction of AVP from the plasma was done using ice-cold absolute ethanol. The samples were centrifuged (2000 rpm, for 15 min), followed by aspiration and evaporation of the ethanolic phase. A radioimmunometric assay (RIA, Peninsula Laboratories, Inc., Belmont, CA, USA) was used according to the instructions provided by the supplier to determine AVP. Results were disregarded in case that the discrepancy between duplicate values was >15%. Data are indicated in ng/l plasma as mean values ± standard deviation. Statistical analysis was executed with StatView (Abacus, Berkeley, CA, USA). Only plasma levels of AVP assayed in blood samples that were drawn from both the cubital vein and the cavernous compartment of the volunteers were statistically evaluated. To compare AVP levels in the systemic and cavernous blood, the Student's t-test was applied. A p value ≤ 0.05 was considered significant.

2.4. Chemicals. NE (Arterenol™) was from Sanofi-Aventis AG (Frankfurt am Main, Germany). AVP was purchased from Bachem Peptides and Biochemicals (Bubendorf, Switzerland). All other laboratory chemicals were from Sigma Chemical Co. (St. Louis, MO, USA), Mallinckrodt-Baker BV (Deventer, The Netherlands), and Merck KGaA (Darmstadt, Germany).

3. Results

3.1. In Vitro Contraction-Response Studies. During the equilibration period, only a few strip preparations generated spontaneous contractile activity. AVP induced concentration-dependent, long-lasting tonic contractions but did not induce phasic contractile activity in the non-stimulated

tissue preparations. The tissue was dose-dependently contracted by physiological concentrations of both AVP and NE in the nanomolar range (0.1 nM–100 nM). In the entire concentration interval examined, the effects of AVP were much greater than were those of NE. It was clearly seen at all concentrations, the mean contractile force evoked by AVP was several-fold greater than that evoked by NE ($p \leq 0.05$). This became most obvious at low concentrations (0.47 ± 0.07 vs. 0.1 ± 0.026 gr at 1 nM and 0.75 ± 0.07 vs. 0.25 ± 0.01 gr at 10 nM). At the highest concentration used (0.1 μM of AVP), the generation of contractile force amounted to 0.85 ± 0.07 gr, whereas the same dose of NE resulted in a tension force of 0.6 ± 0.06 gr. The results are shown in Figure 1.

3.2. AVP Levels in the Systemic and Cavernous Blood of Healthy Males and Patients with ED. In both cohorts, healthy males and ED patients, the sampling of blood had to be terminated during the experimental sequence in some of the volunteers because of pain following insertion of the butterfly needle into the penis or dislocation of the needle. Blood withdrawal from both a cubital vein and the CC at the penile conditions of flaccidity, tumescence, rigidity, and detumescence was commenced in 14, 25, 24, and 17 (out of 25) healthy subjects. In the absence of sexual stimulation, in the phase of penile flaccidity, mean AVP (ng/l plasma) was 5.4 ± 2.7 in the systemic circulation vs. 3.3 ± 2 in the samples aspirated from the CC. With the induction of sexual arousal and the initiation of penile tumescence and rigidity, AVP significantly decreased in the systemic blood to 3.8 ± 3.7 (in the phase of tumescence) and decreased further to 2.9 ± 2.7 (at full rigidity). Following the termination of sexual stimulation, at penile detumescence, mean AVP level in the systemic circulation did not present alterations (remained at 3 ± 2.2). At all penile conditions, no significant changes in AVP were registered in the cavernous blood (see Table 1). At penile flaccidity, the mean AVP plasma level was significantly higher in the systemic circulation than the cavernous blood (see Figure 2(a)). In the cohort of patients with ED, simultaneous blood sampling from the cubital vein and the CC was conducted in 32, 35, and 29 (out of 45) subjects at penile flaccidity, tumescence, and detumescence, respectively. In the patients, at penile flaccidity, prior to the beginning of the sexual stimulation, mean AVP level in the systemic blood was lower than in the blood samples taken from the healthy volunteers (2.5 ± 2.9 vs. 5.4 ± 2.7). Moreover, the course of AVP through the phase of sexual arousal, as indicated by the penile condition of tumescence, did not display transient alterations (see Figure 2(b) and Table 2). In the patients, at penile detumescence, the level of AVP in the penile blood did not differ significantly from the concentration registered in the healthy subjects (3.0 ± 3.4 vs. 2.8 ± 2.7).

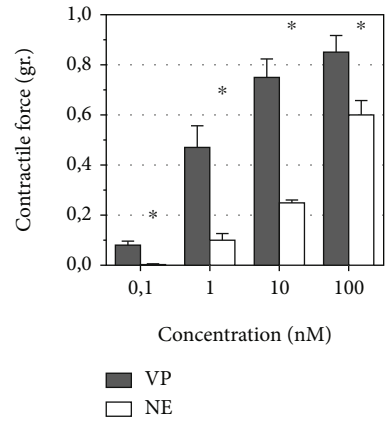

Figure 1: Tissue bath studies: isometric force generation of isolated human corpus cavernosum penis in response to the addition of physiological concentrations of arginine-vasopressin or the alpha-adrenoceptor agonist norepinephrine (NE) (0.1 nM–100 nM). Asterisk indicates that the contraction responses measured following the addition of a distinct concentration of AVP or NE are significantly different from each other ($p < 0.05$).

Table 1: Plasma levels of arginine-vasopressin (AVP) in peripheral and cavernosal blood samples taken from healthy male volunteers during different conditions of sexual arousal, exemplified by the penile conditions flaccidity, tumescence, rigidity, and detumescence.

Penile condition	Blood source	Blood withdrawals commenced	AVP (ng/l plasma)
Flaccidity	CV	14	5.4 ± 2.7
	CC	14	$3.3 \pm 2.0^{*}$
Tumescence	CV	25	$3.8 \pm 3.7^{\S}$
	CC	25	3.4 ± 3.1
Rigidity	CV	24	$2.9 \pm 2.7^{\S}$
	CC	24	2.9 ± 2.6
Detumescence	CV	17	$3.0 \pm 2.2^{\S}$
	CC	17	2.8 ± 2.7

§ indicates that the concentration of AVP is significantly different from those measured in the phase of penile flaccidity. Asterisk (*) indicates that the concentration is significantly different from those measured in the systemic blood. CC: corpus cavernosum; CV: cubital vein.

4. Discussion

The regulation of corpus cavernosum smooth musculature is a complex physiological mechanism where numerous central and local transmitter compounds interact [20]. Aside from the sympathetic and parasympathetic system and the gaseous neurotransmitter nitric oxide (NO), many other non-adrenergic/non-cholinergic (NANC) mediators, such as the endogenous peptides AVP, oxytocin, NPY, substance P, and VIP, have been investigated as to whether they play a significant role in the control of penile vascular and nonvascular smooth muscle. AVP can elicit substantial tonic contractions of isolated corpus spongiosum and corpus cavernosum tissues, these contractions are markedly antagonized by the V1A receptor antagonist SR49059, suggesting that the constriction response is mediated via the activation of this receptor subtype [21]. Based on the results from

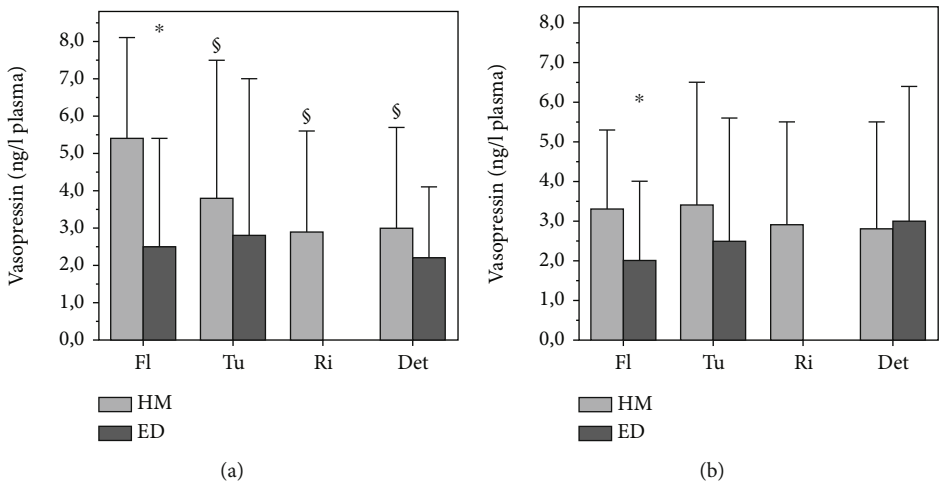

Figure 2: (a, b) Course of arginine-vasopressin (AVP) in (a) the systemic and (b) cavernous blood of healthy men (HM) and in a cohort of patients with erectile dysfunction (ED) at different stages of sexual arousal, exemplified by penile flaccidity (Fl), tumescence (Tu), rigidity (Ri, attained only by the healthy subjects) and detumescence (Det). Data are displayed as mean values ± standard deviation in ng vasopressin/l plasma. Asterisk (*): concentrations are significantly different from each other; §: concentration of AVP is significantly different from the concentration at flaccidity.

Table 2: Plasma levels of arginine-vasopressin (AVP) in peripheral and cavernosal blood samples taken from patients with erectile dysfunction (ED) during different conditions of sexual arousal, exemplified by the penile conditions flaccidity, tumescence and detumescence.

Penile condition	Blood source	Blood withdrawals commenced	AVP (ng/l plasma)
Flaccidity	CV	32	2.5 ± 2.9
	CC	32	2.0 ± 2.0
Tumescence	CV	35	2.8 ± 4.2
	CC	35	2.5 ± 3.1
Detumescence	CV	29	2.2 ± 1.9
	CC	29	3.0 ± 3.4*

Asterisk (*) indicates that the concentration is significantly different from those measured in the systemic blood. CC: corpus cavernosum; CV: cubital vein.

radioimmunoreactivity studies demonstrating the presence of AVP in human cavernous tissue [16], the hypothesis of a local uptake or production/release of the peptide during the different functional conditions of the corpus cavernosum (tumescence, rigidity, and detumescence) seen in conjunction with the male sexual response cycle has been conceived but, up until today, not been proven convincingly [17]. In particular, the potential role of AVP in the pathophysiology of ED still remains to be determined. This prompted us to investigate the courses of AVP through the sexual response cycle in the systemic and cavernous blood of ED patients in comparison to a cohort of healthy males.

In vitro, physiological and pharmacological concentrations of AVP (0.1 nM–0.1 microM) exerted long-lasting contraction of isolated human corpus cavernosum penis; the contraction responses were more potent than those elicited by the alpha-agonist NE. This is in support of the hypothesis that AVP acts as an antagonist of the relaxation of cavernous smooth muscle, which is the paramount event in the erection process. In the in vitro experiments, penile erectile tissue from transsexual male patients, who had received hormonal replacement (androgen deprivation) prior to gender reassignment surgery, was used. This raises the question as to whether such tissue is suitable to be applied to tissue bath studies. Indeed, it has been demonstrated that the physiological responses of isolated corpus cavernosum obtained from normal men vs. men under hormonal treatment to compounds known to either exert or counteract the contraction of vascular and nonvascular smooth muscle do not necessarily differ considerable greatly [22].

In the healthy subjects, a marked decline in systemic AVP was seen at the beginning of sexual arousal, when the flaccid penis became tumescent and rigid. No further decline was registered when the erected penis returned to the flaccid state. This could be interpreted in terms of that the drop in systemic AVP levels, potentially caused by the so-called Gauer-Henry reflex [23], facilitates the relaxation of penile vascular and nonvascular smooth muscle in order to achieve penile rigidity, thus preventing that the several-fold increase in blood flow into the penis to induce tumescence and full erection yield to an elevation in AVP in the cavernous compartment [24]. In contrast, in the blood drawn from the penile lacunar space, no relevant changes in AVP levels were detected through the different functional conditions of the penis. At penile flaccidity, the mean AVP level in the systemic blood was higher than in the blood aspirated from the penis (corpus cavernosum). In the patients with ED, the AVP concentration in the systemic circulation was overall lower than in the healthy subjects. In fact, it is well known that age is one of the risk factors for ED, thus, the findings from the group of patients could be possibly explained by the fact that the individuals were of older age (mean: 52 years vs. 25 years in the cohort of healthy males)

and exhibited some age-related comorbidities, such as hypertension, atherosclerosis, obesity, and diabetes. With aging, hypothalamic, and neurohypophyseal activity may alter, thereby also affecting at the level of the genome, transcriptome, or peptidome the production and/or secretion of AVP [25]. Moreover, no decrease in the plasma concentration of the peptide was registered with the beginning of sexual arousal. This observation might reflect changes in pituitary function that have been shown to be associated with ED and may result in hypogonadotropic hypogonadism, growth hormone deficiency, or hypocorticolism [26, 27]. Since the pituitary gland serves as storage for AVP, any pathological condition affecting pituitary function can, as a result, also lead to disturbances in the control of circulating levels of AVP. However, this should take into consideration that it is yet not well understood as to whether the measurement of peripheral concentrations of neuropeptides can serve as an index for central concentrations [28, 29]. As seen in the healthy subjects, the course of AVP in the cavernous compartment of the ED patients did not alter during the different penile conditions, either with the initiation or with the termination of sexual stimulation. In conclusion, our study revealed differences in the courses of circulating AVP through the stages of sexual arousal in the systemic blood of healthy males and men presenting with ED. However, it remains to be elucidated further as to whether, in the aging male, age- or disease-related disturbances in the production, and/or release of AVP might be a critical factor contributing to the manifestation of ED. Although it has been shown earlier that the cavernous compartment possesses paracrine physiological properties [30], based on our findings, it seems unlikely that the corpus cavernosum is a major site of synthesis, release, or degradation of AVP.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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