

The pharmacology of penile smooth muscle

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Introduction

To maintain the penis in the flaccid state, the penile vasculature and the smooth muscle of the corpus cavernosum is kept partly contracted. This state of contraction is produced not only, but probably not solely, by noradrenaline released from adrenergic nerves acting on postjunctional α -adrenoceptors. However, nonadrenergic, noncholinergic [NANC] mechanisms may contribute [1]. Penile erection is associated with parasympathetic activity, which decreases the effects of contractile factors and produces relaxation. It is, however, unlikely that the relaxation-producing transmitter is acetylcholine, because the effects of atropine on erections induced by stimulation of the pelvic or cavernous nerves (varying [1, 2], atropine lacks effect on erections provoked by visual or tactile stimulation in man [3], and muscarinic receptor antagonists have a poor effect on relaxations induced by nerve stimulation in isolated erectile tissue [4, 5]. Much of the present research in the field is therefore focused on NANC mechanisms, as evidenced by recent reviews [1, 2, 6].

Below, some of the factors believed to contribute to the regulation of penile smooth muscle tone are briefly discussed.

Maintenance of penile smooth muscle tone

During the flaccid state, there is a high resistance to penile blood flow due to contraction of the helicine arteries and of the corporeal smooth muscle cells [7]. α -adrenoceptor stimulation seems to play a significant role in this respect, and in the corpus cavernosum, the α_1 -adrenoceptor is the functionally dominating subtype. In this tissue, three subtypes of α_1 -adrenoceptor mRNA were recently identified (α_{1A} , α_{1B} , and α_{1C}), the α_{1A} - and α_{1C} -subtypes predominating [8]. As mentioned above, noradrenaline is probably not the only factor responsible for long- and short-term maintenance of penile smooth muscle tone.

Myogenic activity. Strip preparations of human corpus cavernosum may exhibit spontaneous contractile activity, the occurrence of which varies in different investigations between approximately a few % and 100% [1]. This activity, which is not affected by phallotoxin, atropine, or phentolamine, seems to have a myogenic origin, and is the result of synchronized mechanical activity in individual muscle cells. Anoxia eliminated spontaneous contractile activity, and reduced basal tension, suggesting that the tone is dependent on the state of corporeal oxygenation [9].

L-type calcium channels have been identified in corporeal smooth muscle cells [10], and removal of extracellular calcium and addition of calcium antagonists can abolish spontaneous contractile activity. This can be achieved also with potassium-channel openers, which act by causing hyperpolarization, thereby decreasing the opening probability of the L-type calcium channels [11-13]. The smooth muscle cells of the corpus cavernosum contract and relax in a rapid and synchronous way, and the electrical activity of the human corpus cavernosum in vivo, as revealed by electromyographic studies, is well synchronized [14, 15]. It has been suggested that corporeal smooth muscle cells behave as a functional syncytium [10, 16], and a mechanism involving gap junctions, by which local neural and hormonal stimulation can be rapidly propagated in

corporeal tissue was recently postulated [10, 17-19]. Human corporeal smooth muscle cells are well coupled with respect to intercellular diffusion of current carrying ions and of second messengers, such as calcium, cyclic nucleotides, and inositol triphosphate [10, 16]. Gap junctions might also modulate the passive spread of electrotonic current flow, as well as regulate regenerative electrical events. Several gap junction proteins, referred to as connexins, have been identified. In human corpus cavernosum, connexin43 is the predominant, though perhaps not the only, type [17].

The extent of junctional communication is voltage dependent, i.e., the intercellular spread of currents through gap junctions is sensitive to the difference in membrane potential existing between a pair of adjacent cells [10]. The resting potential of cultured corporeal smooth muscle cells was reported to be -40 to -50 mV [10, 16]. In enzymatically isolated smooth muscle cells from the human corpus cavernosum, however, the membrane potential was considerably higher than in the cultured cells, and a mean value of 72 mV was recorded [20]. Furthermore, cultured cells from human corpus cavernosum are acontractile [21], but in enzymatically isolated corporeal cells the contractile ability is maintained [22]. Therefore, it seems reasonable to assume that there are significant differences in excitation-contraction coupling mechanisms between enzymatically isolated and cultured corporeal cells.

The electrophysiological basis for and the physiological significance of the spontaneous contractile activity in isolated corpus cavernosum tissue is unknown. Such activity may be of importance *in vivo* and contribute to the maintenance of tone and to the mechanisms of detumescence.

Prostanoids. The myogenic activity in corporeal tissue may, at least partly, result from the generation and/or presence of a stable cyclo-oxygenase product, since inhibitors of prostaglandin (PG) synthesis were shown to reduce spontaneous contractions and to decrease resting tone [1]. Human corpus cavernosum has the ability to synthesize various prostanoids including thromboxane A_2 and $PGF_{2\alpha}$ [23-25], and contains an inactivating enzyme, prostaglandin 15-hydroxydehydrogenase [26]. Several prostanoids were shown to contract human isolated corpus cavernosum, including the stable thromboxane A_2 analogues U44069 and U46619 and $PGF_{2\alpha}$ [27-29]. The main contraction-mediating prostanoid receptor in this tissue seems to be a thromboxane A_2 -sensitive receptor, although the presence of additional contraction-mediating prostanoid receptors cannot be excluded [28-29]. Also PGI_2 , a well-known inhibitor of platelet aggregation [30-31], is synthesized by human cavernous tissue [24, 25]. However, in contrast to the vasodilating effect of PGI_2 in other vascular preparations, Hedlund and Andersson [27] demonstrated this prostanoid to contract penile erectile tissue. In concert with this, PGI_2 injected intracavernously in pigtailed monkeys caused a large reduction of the cavernosal compliance owing to smooth muscle contraction [32]. However, there was no change in cavernosal arterial blood flow. There is thus no unequivocal evidence that PGI_2 directly contributes to regulation of corporeal smooth muscle tone. Nevertheless, even if it does not, this prostanoid may well be of importance during penile enlargement and blood stasis, counteracting local thrombosis formation.

Endothelins. Endothelins (ET) are among the most potent contractile agents known today [33]. Three structurally and pharmacologically distinct isotopes have been demonstrated, ET-1, ET-2, and ET-3 [34-36]. Cultured endothelial cells from the human corpus cavernosum, but not non-endothelial cells, were shown to express ET-1 mRNA [37]. Binding experiments revealed at least two distinct ET receptors in corporeal membranes, one type with high affinity for ET-1 and -2 and low affinity for ET-3, and another, less abundant, with high affinity for ET-1, -2, and -3 [37].

Significant amounts of ET-like activity were measured with radioimmunoassay in the supernatants of endothelial cells in culture [37]. By means of an ET-1 monoclonal antibody, ET-like immunoreactivity was found to be localized intensely in the endothelium, and to a less degree, in the cavernous smooth muscle from humans [37]. Thus, the capability to synthesize and release ETs appeared to be specific for endothelial cells. By autoradiography, it was shown that there are numerous binding sites for ET-1 in human penile erectile tissue, and that these are generally not confined to a single structure or region, but are distributed rather uniformly [38]. ET-1 potently induced slowly developing, long-lasting contractions in isolated corpus cavernosum [37-41]. Contractions were evoked also by ETs- 2 and -3, although these peptides had a lower potency than ET-1 [37].

The mechanisms of ET-induced contraction of penile tissues and vessels have not been fully elucidated. In corpus cavernosum, the contractions evoked by ET-1 were attenuated by the calcium antagonist nimodipine and greatly reduced, but not abolished, in calcium-free medium [38]. Thus, the contractions induced by ET-1 are mainly, but not exclusively, dependent on extracellular calcium, and part of the calcium-influx is through dihydropyridine-sensitive calcium channels. The mechanism mediating ET-1 contraction in calcium-free medium is not clear. ET-1 was shown to induce hydrolysis of phosphoinositides in a concentration-dependent manner in rabbit corpus cavernosum tissue [40]. However, whether or not ET-1 activates phospholipase C also in the human corpus cavernosum remains to be established.

Available evidence thus suggests that ET-1 can act through three different mechanisms in corpus cavernosum: by increasing the influx of Ca^{2+} from the extracellular space, by releasing calcium from intracellular stores, and by increasing the sensitivity for calcium of the contractile machinery. Secretion of ET-1 from vascular endothelial cells seems to be regulated at the level of mRNA transcription [33], and also in cultured endothelial cells from human corpus cavernosum, ET-1 mRNA is expressed [37]. Using various endothelial cell preparations, Nakamura et al. [42] demonstrated that ET-1 is secreted by exocytosis without prior concentration and storage in secretory granules. Furthermore, it has been shown that the slow continuous release of ET-1 can be stimulated by factors such as arginine vasopressin, angiotensin [43], thrombin [44], and hypoxia [45]. It may therefore be speculated that the release of ETs in corpus cavernosum is regulated at the level of synthesis rather than secretion, and that high concentrations of ETs may be reached locally contributing to sustained smooth muscle tone. Thus, although the exact role of ETs in the control of penile smooth muscle tone has to be established by experiments in vivo, e.g., by the use of ET-receptor antagonists, much of the available information suggests that the peptides may be of importance for the mechanisms of detumescence and flaccidity.

Neuropeptide Y. NPY has been shown to be localized with noradrenaline in adrenergic postganglionic neurons and participate with noradrenaline in the vasoconstrictor response of many blood vessels [46-47]. The peptide has also been demonstrated in human penile vasculature and erectile tissue [48-53]. Adrian et al. [49] found moderately high concentrations of NPY in the human corpus cavernosum, but nevertheless, they suggested that NPY could be intimately involved in the control of erection. Wespes et al. [50] studied the distribution of NPY-containing nerves in the human penis. They found a concentration of fibres in the inner part of the adventitia close to the media of the arterial and venous vessels and among the intracavernous smooth muscle cells, and speculated that the peptide could act as a neurotransmitter or neuromodulator, especially during detumescence. The observation that in rats chemical sympathectomy, but not α -adrenoceptor blockade, increased emptying rate, made Giuliano et al. [54] to postulate that a NANC transmitter colocalized with noradrenaline exerted a contractile effect on

penile veins, and that NPY may be this neurotransmitter. Despite lack of experimental evidence, NPY was thus suggested to take part in detumescence. Crowe et al. [52] arrived at a similar conclusion based on immunohistochemical studies only. In addition, they suggested that NPY may have a prolonged contractile response to nerve stimulation and a potentiating effect of the vasoconstriction caused by noradrenaline in this tissue. Speaking against this statement, NPY did not enhance the actions of noradrenaline in rabbit corpus cavernosum [55].

If NPY is a neurotransmitter involved in detumescence, a contractile effect on erectile tissues would be expected. Hedlund and Andersson [56] found no effects of NPY in corpus cavernosum preparations studied at basal tension level or contracted by noradrenaline, nor were electrically induced contractions affected. Kirkeby et al. [53] found that 6 out of 8 preparations of normal corpus cavernosum did not respond to NPY; two of the preparations responded with phasic contractions; the mean contraction amounted to only 18% of the contraction induced by high K^+ .

The reported effects of NPY on human corpus cavernosum are thus inconsistent, and experimental evidence supporting that a contractile mechanism involving NPY may be operating in man is still lacking.

Arginine vasopressin. In human cavernous tissue, AVP-like activity could be demonstrated by radioimmunoassay in concentrations up to 10 times those circulating in plasma, suggesting that the peptide was either taken up and stored and/or synthesized locally [57]. Furthermore, AVP was found to contract isolated human corpus cavernosum in a potent and concentration-dependent manner, effects that could be inhibited by AVP-antagonists [56, 57]. AVP antagonists, on the other hand, had no effect on electrically induced contractions in corpus cavernosum preparations [57], indicating that AVP is not released on electrical stimulation in amounts that can affect the smooth muscle directly and/or influence the response to released noradrenaline. However, considering that relatively high amounts of AVP-like activity were detected in corpus cavernosum and that AVP contracted this tissue probably through specific receptors, a modulatory function of AVP cannot be excluded. Furthermore, trophic influences such as the nerve-growth promoting effect described by Brinton and Gruener [58] should be considered.

Penile smooth muscle relaxation

Parasympathetic activity seems to play a significant role in penile erection, although existing data concerning the effects of acetylcholine and different muscarinic receptor antagonists are contradictory. However, it is important to remember that parasympathetic activity is not equivalent with the actions of acetylcholine; other transmitters may be released from cholinergic nerves. Thus, there are at least three mechanisms by which parasympathetic activity may contribute to penile smooth muscle relaxation: 1) the neuronal release of noradrenaline may be inhibited by stimulation of prejunctional muscarinic receptors, 2) endothelium-derived relaxant factors may be released through stimulation of postjunctional muscarinic receptors, and 3) NANC relaxant factors may be released directly from parasympathetic nerves.

Nitric oxide. The vascular endothelium can generate factors that relax smooth muscle cells, and one of these factors, the endothelium-derived relaxing factor [EDRF], was first described in 1980 [59], and was subsequently identified as nitric oxide [NO], synthesized from L-arginine by an endothelial NO synthase [NOS; 60-62]. NO can be released from endothelial cells in response to several agents including acetylcholine [63]. In isolated preparations of the human corpus cavernosum [5, 64-66] and spongiosum [4],

pronounced relaxations were found in response to muscarinic receptor stimulation and to electrical stimulation of nerves. Several investigators have shown that both these effects involve the release of NO, or a NO-like substance. Analogs of L-arginine, like L-NAME [N^G -nitro-L-arginine methyl ester], L-NMMA [N^G -monomethyl-L-arginine], and L-NNA [N^G -nitro-L-arginine], effectively inhibit both endothelial and neuronal NOS, and in vitro they inhibit the relaxation of cavernous tissue caused by muscarinic receptor stimulation and electrical stimulation of nerves [67-77].

Both the endothelium and/or the nerves innervating the corpus cavernosum may theoretically be the source of the NO involved in erection. Burnett et al. [78] recently identified NOS in the human cavernous nerves and their terminal endings within the corpora cavernosa, in the branches of the dorsal penile nerves and nerve plexuses in the adventitia of the deep cavernous arteries. NOS localized to the endothelium and to the smooth muscle of the human corpora cavernosa, has so far not been clearly demonstrated by immunohistochemistry. However, Keast [79], using NADPH diaphorase staining to demonstrate NOS in the rat penis, found the enzyme both in endothelial cells lining many blood vessels and within the cavernous spaces. NOS demonstrated in rat and rabbit corpus cavernosum was shown to display substantial activity, as monitored by the ability to convert [3H]arginine to [3H]citrulline [80, 81]. In rabbit corpus cavernosum, the enzyme present was shown to be a cytosolic, constitutive isoform of NOS [81], like that found in brain neuronal tissue [62]. In contrast, the endothelium-derived NOS is primarily membrane-bound [82]. This suggests that the most important source of NO in penile tissue is neuronal. Supporting this view, isolated cavernous tissue responded with relaxation to electrical stimulation of nerves after destruction of the endothelium, while responses to acetylcholine, bradykinin, and SP were abolished [5, 64, 65]. Furthermore, in anesthetized dogs, intracavernous injection of CHAPS to destroy the sinusoidal endothelium, abolished the erectile response to acetylcholine, but only partially inhibited the response to electrostimulation [83].

NO, and vasodilators acting through NO, like nitroglycerin, sodium nitroprusside, S-nitroso-N-acetylpencillamine, and linsidomine, which all cause concentration-dependent relaxation of corpus cavernosum [67-71, 84, 85], have been shown to stimulate soluble guanylate cyclase, leading to an increase in the tissue levels of cyclic GMP. The cyclic GMP increase produces relaxation of smooth muscle cells presumably by reduction of the free calcium concentration, and several mechanisms have been suggested to mediate this effect [63]. Cyclic GMP is degraded intracellularly by different phosphodiesterases (PDEs), and in human corpus cavernosum, three different PDE isoenzymes have been found [86]: PDE III (cyclic GMP-inhibited), PDE IV (cyclic AMP specific) and PDE V (cyclic GMP specific). Consequently, in this tissue, the relaxant effects of electrical stimulation [67, 68, 71, 87] and linsidomine [88] can be enhanced by selective inhibition of the cyclic GMP phosphodiesterases. Furthermore, spontaneous contractile activity and noradrenaline-induced contractions were opposed by different PDE inhibitors, quazinone (PDE III inhibitor) being the most potent [86, 88].

In rats, intracavernous injection of different drugs known to act at different levels of the cyclic AMP and cyclic GMP pathways, revealed that neither cyclic AMP, nor drugs that stimulate adenylate cyclase activity, induced any change in intracavernosal pressure [89]. Intracavernosal injection of cyclic GMP and nitroprusside, on the other hand, caused dose-dependent changes in intracavernous pressure that could be inhibited by the guanylate cyclase inhibitor methylene blue, and it was concluded that cavernous smooth muscle relaxation in the rat is mediated by activation of guanylate cyclase, and that the cyclic AMP system apparently has no important role [89]. Supporting this, Dahiya et al.

[90] showed that penile erection induced by neurostimulation and sodium nitroprusside in the dog is associated with increased levels of cyclic GMP, but not cyclic AMP. In monkeys, both guanylate cyclase and adenylate cyclase may be involved in cavernous smooth muscle relaxation, with cyclic GMP being the predominant intracellular second messenger [91]. The involvement of cyclic AMP in penile erection in humans remains to be established, but cannot be excluded (92).

L-NNA augmented the contractions induced by electrical field stimulation or by noradrenaline, but had little effect on resting tension [69], suggesting that passive stretching of cavernous tissue is not sufficient to induce NO release, but that continuous release of NO may occur during active contraction. NO may therefore be involved also in the control of penile blood flow during the flaccid state. Interestingly, NO production, but not the ability of the smooth muscle to respond to NO, seems dependent on the oxygen tension [93-94]. Thus, electrically-induced relaxations were progressively inhibited as a function of decreasing oxygen tension at pO_2 values below 50 mm Hg, and markedly attenuated at oxygen tensions measured in the flaccid state. This would mean that the low oxygen tension in the flaccid state is associated with a decreased activity of the NOS, thereby reinforcing the mechanisms responsible for the maintenance of a high penile smooth muscle tone. Furthermore, during erection with subsequent blood stasis, NOS activity would gradually decrease, thereby promoting detumescence and preventing the penis to be damaged due to prolonged erection.

Available *in vitro* results obtained in isolated penile tissues thus suggest that the penile L-arginine/NO system is essential for normal erection. There is accumulating *in vivo* data supporting this view. For instance, erections induced by stimulation of the cavernous nerves in anesthetized rabbits could be dose-dependently inhibited by intracorporeal injection of L-NNA, inhibiting NOS [95]. In the pithed rat, L-NNA attenuated the corporeal response to spinal stimulation [96-97], and in the intact rat, *i.v.* L-NNA in low doses (1-5 mg/kg) inhibited erection induced by stimulation of the cavernous nerves [82]. In dogs, L-NNA blocked pelvic nerve-stimulated erections. This effect was reversed by intracavernous injection of L-arginine, inhibited by methylene blue and enhanced by a cyclic GMP phosphodiesterase inhibitor [98]. In addition, intracavernous injection of cyclic GMP caused erection in 13 out of 15 impotent men [99], and, in two patients with priapism, methylene blue administered through the same route produced detumescence giving further support for a role of the L-arginine/NO pathway in penile erection [100]. On the other hand, erection in male volunteers were not associated with any measurable levels of nitric oxide metabolites in cavernous or peripheral blood [101]. However, this may be due to methodological difficulties when measuring such drug levels.

It may be speculated that possible pathological changes of the L-arginine/NO pathway in penile erectile tissue are predisposing for, or even are the major causes of, erectile dysfunction of certain etiologies. For instance, in isolated corpus cavernosum from diabetic patients with impotence, both neurogenic [102-104] and endothelium-dependent [102, 104] relaxation was impaired. This was associated with a lack of NO production, measured as the formation of nitrite, and not to inability of the smooth muscle to relax [103]. Hypercholesterolemia was also found to impair endothelium-mediated relaxation of rabbit corpus cavernosum smooth muscle [105]. The mechanism of this effect is not known, but did not seem to involve cyclooxygenase products or the ability of the smooth muscle to react to stimulation by a NO donor (nitroprusside).

If, in some cases, an impairment of the L-arginine/NO pathway is the causative factor of erectile dysfunction, it would be rational to treat the condition on the basis of the underlying disorder. In cats, NO-donors [106] and sodium nitroprusside [107] injected

intracavernously caused erectile responses. In men with erectile dysfunction, intracavernous injection of both the NO-donor linsidomine [109-111] and cyclic GMP [99] produced erection. Also topical nitroglycerin has been shown to have a positive effect on erection in impotent men [112-115]. However, when sodium nitroprusside was given intracavernously to three impotent patients, only mild tumescence was produced, but severe hypotension was obtained [116]. Obviously, nitric oxide donors may be used to illustrate the importance of the L-arginine/NO pathway in the erectile process, but the clinical potential of these drugs in the treatment of impotence has to be further evaluated.

There is thus good experimental evidence for the assumption that neurogenic nitric oxide is an important mediator in penile erection. It is therefore surprising that mice that lack the neuronal NOS are both viable and fertile [117], which means that in these animals, there are other possibilities to produce penile erection. It should be remembered, however, that these mice, generated by homologous recombination, may have other possibilities to develop compensatory mechanisms than individuals in whom a dysfunction of the L-arginine/NO system is produced in adult life.

Vasoactive intestinal polypeptide and related peptides. In vitro studies of strips of human corpus cavernosum tissue have shown that VIP has an inhibitory and relaxation-producing effect. The effect on spontaneous (myogenic) contractile activity and on electrically induced contractions was pronounced; it was less obvious on noradrenaline-contracted preparations [1]. There are many immunohistochemical studies demonstrating VIP in autonomic nerves in the smooth muscle of the human penis [4, 48, 118-124], and the density of VIP-containing nerves was described as exceeding that of adrenergic nerves [51]. VIP has been found in high concentrations in the erectile tissue [119, 122, 126-128]. VIP was shown to be co-localized with NPY in the cavernous tissue and helicine arteries of the monkey [46]. Also VIP and acetylcholine seem to be co-localized in parasympathetic pathways to the penis of animals and man [121, 129, 130]. In noradrenaline-contracted human corpus cavernosum tissue, the relaxation induced by acetylcholine and VIP together was found to be no greater than that obtained by acetylcholine or VIP given separately [131], and it was therefore suggested that the demonstrated co-existence of acetylcholine and VIP has no functional significance. On the other hand, in dogs, Takahashi et al. [132] demonstrated that simultaneous intracavernous injection of VIP and acetylcholine produced a synergistic effect, and it was suggested that the two agents may play a co-operative role in canine penile erection [132]. Thus, even though VIP seems to be of no importance for the action of acetylcholine in human erectile tissue, this peptide may well interact with the L-arginine/NO system. Supporting such a view, VIP and NOS were found to be co-localized in nerves of the human corpus cavernosum [133].

It is generally held that VIP and related peptides relax smooth muscle by stimulating adenylate cyclase and increasing the intracellular concentration of cyclic AMP [134, 135]. However, in short term cultures of human corpus cavernosum smooth muscle cells had no effect on cyclic AMP production [92]. The reason for this is unclear.

Pilot studies in man have demonstrated a marked release of VIP during tumescence and erection produced by visual sexual stimulation, by intracavernous injection of papaverine, or by intracavernous infusion of saline [136-137]. In patients with either predominantly organic or predominantly psychogenic impotence, Kiely et al. [138], measuring cavernosal and peripheral VIP concentrations during erection induced by a variety of vasoactive compounds, found no increase in cavernosal VIP concentration. Gu et al. [125] found that in patients with impotence, VIP containing nerves were depleted, and that the extent of the depletion broadly reflected the severity of erectile dysfunction,

irrespective of its etiology. In diabetic patients with impotence, a marked reduction of VIP-like immunoreactivity in nerves associated with the cavernous smooth muscle was reported [121]. These findings suggested that VIP is a principal neurotransmitter involved in penile erection, and that depletion of the peptide may play a key role in the development of impotence. However, relaxant effects of VIP *in vitro*, and presence of VIP and VIP-containing nerves in penile erectile tissue, do not automatically prove that this peptide is of physiological importance in penile erection. Speaking against such a role, VIP-antiserum [139] and α -chymotrypsin [140], reducing or abolishing the relaxant effect of exogenous VIP on isolated human corpus cavernosum tissue, had no effect on relaxations induced by electrical stimulation of nerves. This would indicate that there is no neuronal release of VIP, at least not in the *in vitro* situation. In addition, VIP did not produce erection when injected intracavernosally in healthy volunteers [141], and in impotent men [139, 142, 143].

Peptide histidine methionine (PHM) is derived from the same precursor as VIP, and was shown to be localized to the nerves in close relation to bundles of smooth muscle and around arteries in the human corpus cavernosum and in circumflex veins [119, 144], and so have the VIP-related peptides pituitary adenylate cyclase activating peptide (PACAP), and helospectin [134, 135]. A role for these peptides as neurotransmitters and/or neuromodulators cannot be excluded, but conclusive evidence is lacking. In strips of the human corpus cavernosum contracted by noradrenaline, PHM caused only a small (10%) relaxation. PACAP-27 and helospectin-1 were found to have similar relaxant effects as VIP, both on noradrenaline-induced and electrically evoked contractions [134-135].

Even if NO probably is the most important vasodilator in the process of erection (see above), this does not exclude that other agents released from nerves may have a modulatory function. However the roles of VIP and related peptides as neurotransmitters and/or neuromodulators in the nervous control of penile erection have to be established.

Calcitonin gene-related peptide. CGRP-like immunoreactivity has been demonstrated in nerves of the human corpus cavernosum [145]. CGRP is known to be a potent vasodilator in a variety of human blood vessels, where it is believed to produce an endothelium-dependent relaxation [146]. In monkeys, CGRP given intracavernously increased cavernous arterial flow, induced cavernous smooth muscle relaxation, and venous outflow occlusion [147]. Also in man, erectile responses have been recorded after intracavernous injection of the peptide [145]. However, *in vitro*, CGRP had little relaxant effects on strips of human corpus cavernosum contracted by noradrenaline or by electrical field stimulation [148]. If the peptide has a role in normal penile physiology remains to be established, but even if not, this does not exclude that it may be useful for therapeutic purposes [145, 149].

Prostaglandin E. The precise role of prostanoids in the physiology of normal erection is not known. However, the widespread use of intracavernosally injected PGE₁ for treatment of erectile dysfunction [150, 151], has renewed the interest in both the physiological functions of prostanoids in penile erection and their mechanism of action when used therapeutically [152]. The PGE₁ receptors in cavernous tissue from humans, monkeys, and dogs have been investigated by radioligand binding [153]. In tissue from humans and monkeys, but not dogs, PGE₁ receptors were demonstrated. Correspondingly, intracorporeal injection of PGE₁ resulted in erection in humans and monkeys, but not in dog.

PGE₁ effectively relaxed human trabecular tissue contracted by noradrenaline or PGF_{2 α} [27]. Also PGE₂ had a certain relaxant effect; however, at high concentrations

contractions were observed [27]. PGE₁ has been shown also to inhibit release of noradrenaline from penile adrenergic nerves [154], which may contribute to its relaxant action when used for treatment of erectile dysfunction. If such an action is of importance physiologically remains to be demonstrated.

In corpus cavernosum tissue, the signal transduction pathway for PGE₁ may be through adenylate cyclase and cyclic AMP formation [92]. In enzymatically isolated smooth muscle cells from the human corpus cavernosum, patch-clamp analysis and simultaneous monitoring of the intracellular calcium concentration, suggested that extracellularly applied PGE₁ induced smooth muscle relaxation by inhibition of voltage dependent L-type calcium channels and a subsequent decrease of the intracellular calcium concentration [20].

Adenosine triphosphate and adenosine. ATP and other purines were shown to decrease both basal tension and phenylephrine-stimulated tension in rabbit corpus cavernosum [155-157]. Also in the canine penile artery, ATP was found to produce relaxation [158]. The response to ATP was more pronounced than that produced by bethanechol in the same tissue, and was independent of the endothelium [155-156]. It was therefore suggested that ATP is a NANC transmitter in the corpora cavernosa, and that purinergic transmission may be an important component involved in the initiation and maintenance of penile erection [156]. However, none of the purines tested facilitated or inhibited the response of corpus cavernosum to electrical field stimulation, and therefore their role may be in the modulation of erection rather than as a neurotransmitter of erection [157].

ATP injected intracavernously in dogs, was found to produce increases in intracavernous pressure and erection [159]. This effect, which was unaffected by atropine and hexamethonium, could be obtained without changes in systemic blood pressure. Also adenosine produced full erection on intracavernous administration in dogs [160]. The roles of ATP or adenosine in the physiological mechanisms of erection in man remain to be established.

Conclusion

The changes in blood flow, intracavernosal pressure, and penile volume that occur in the erectile process are extremely complex, and require a high degree of coordinated control for normal function. Coordination occurs at many peripheral levels in addition to those demonstrated in the higher centers in the brain and spinal cord. The multiplicity of putative transmitters present in corpus cavernosum and in perivascular nerves, require further investigations, as do the interactions between transmitters and neuromodulators at the neuromuscular junction. Such information is necessary to provide a basis for future clinical ways of treating erectile dysfunction.

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Discussion - THE PHARMACOLOGY OF PENILE SMOOTH MUSCLE

J. Herbert

I have a question that has to do with nitric oxide synthase. It has, as you demonstrated, a very potent effect on components of the penile contractile mechanism. The enzyme has at least two isoforms, one constitutive and one inducible. Clearly, the inducible one might be of particular interest in the context of variations in the penile function. Do you know whether there is much of this second isoform in the penis?

K.E. Andersson

What has been demonstrated immunohistochemically and also biochemically is that in the penis there is the constitutive type of enzyme, but it is only normal tissue that has been investigated, and I think that both the endothelial form and the neurogenic form, which are clearly separable, can be demonstrated there. The possibility that there is the inducible form exists, particularly in conditions where you have some disorders of penile function. A problem is that we have not had any immunohistochemical method for demonstrating the inducible form until recently and I do not know of any biochemical demonstration of the inducible.

M. Murphy

What is the evidence for arginine vasopressin as a contractant of the penile erectile tissue? Before you answer the question I would like to comment about circulating oxytocin. Normally oxytocin is released maximally at orgasm and then the release from the neurohypophysis stops abruptly. In man we have been able to block this release with no effect on penile function. There are other effects but they are not on erection. We found that vasopressin is released during sexual activity but stops being released prior to ejaculation and so blood levels have returned to normal at orgasm.

K.E. Andersson

It was by accident that we found that arginine vasopressin has this potent effect on the penile vasculature and also that the content was so high. We were trying to find

some kind of effect for the drug because it is known that one of the oxytocin effects is the priming of the peripheral tissues for the sexual responses, but we did not find that oxytocin had any potent effect on human penile erectile tissue. It has a small contractant effect at high concentration, but vasopressin in contrast has these tremendous effects. My speculation was that since oxytocin levels are high vasopressin should also be high and that could contribute to detumescence.

M. Baum

I was surprised to hear you say that neurogenic nitric oxide synthase-knockout mice were fertile

K.E. Andersson

In the paper that I mentioned the authors reported that there was no neurogenic nitric oxide synthase demonstrable in the peripheral tissues, which is an interesting thing. I think this focuses on the representativity of knockout mice for a situation in the adults. Neonatally or very early during development you can mobilize other factors and to me it would be very strange that such an essential function as reproduction could be dependent on one single molecule.

G. Wagner

If I may continue along the lines with vasopressin and oxytocin, as a matter of fact, quite a large number of coital functions in the Western world are done under an alcohol level which actually blocks the release of ADH and oxytocin. I do not know whether it is worth looking into it seriously because these are completely knocked out with about 2 mg/g of alcohol in the blood.

M. Murphy

Alcohol also blocks apomorphine and oxytocin induced erections when microinjected into the PVN in the rat. In contrast, alcohol does not affect spinal mechanisms in the way that was previously thought so we need to look for another site of action and oxytocinergic neurons may well be where we will find it.

J.G. Pfaus

Actually alcohol gets us into another interesting question. Like clomipramine, alcohol also delays ejaculation and in rats we can see the development of a very robust tolerance to this effect if the alcohol is administered prior to sexual activity. I wonder what the efficacy would be of drugs that are taken on demand to treat particular types of sexual dysfunction. Do we run the risk of tolerance developing contingently to the effects of these drugs on behaviour? Certainly that happens with alcohol but I do not know if it happens with clomipramine.

J.T. Clark

I think that the effects of alcohol in rats are totally dependent upon whether it is administered chronically or PRN or in acute administration. If we put 10% alcohol in the drinking water or in a liquid diet with 6% alcohol the rats mate perfectly well. There is really no change in any of the parameters of mating behaviour although their erectile reflexes are down, so we have another context-specific effect of alcohol. It is not dependent upon being sexually experienced when they start.

J. Bancroft

Alcohol is a sedative and I would like to know whether alcohol differs in its effects from other sedatives like barbiturates. Can anybody comment on that?

J.G. Pfaus

Obviously barbiturates can reduce sexual function if given in an acute way. I do not know if there are chronic effects and if one gets tolerance to those effects but it certainly raises some issues. In humans it is easy to see situations in which alcohol simply inhibits and it seems to inhibit everything in a progressive way. We also observe disinhibitory effects leading people to engage in very stupid types of behaviour. In rats, when you train them not to copulate, you can see disinhibitory effects of low doses of alcohol. If you do not train them not to copulate, or if you simply administer the alcohol in normal copulatory situations, you only ever see evidence of inhibition. So I wonder if some of the effects that Shakespeare referred to on the stimulation of desire but the disruption of performance have to do with the presence or absence of inhibition to

begin with.

J. Stewart

I would just like to mention out a piece of data that I found very interesting in relation to alcohol. It was reported in Nature this year that women given alcohol show significant rises in testosterone. This does not happen in men. And the effect is very significantly exaggerated in women on the contraceptive pill. I wonder if anyone else had seen this and could comment on that.

J. Bancroft

This is a very interesting study but very difficult to interpret. I think we should consider that the pattern of androgen release is different in women and men in important ways because in women the adrenal contribution is proportionally greater so there may be different mechanisms.

J.R. Heiman

Has anyone tracked changes in testosterone when contraceptives are started?

J. Bancroft

Yes. There are two mechanisms involved: oral contraceptives tend to block follicular ovulation and consequently they reduce follicular development and therefore the mid-cycle rise in testosterone tends to be blunted, and also they stimulate sexual hormone binding globulin so you get an increase in SHBG and the general tendency is for a substantial reduction in free testosterone in women on combined oral contraceptives.

B.D. Sachs

We saw evidence yesterday, both in humans and rats, that penile erection is possible and can be intense with very low testosterone or no testosterone at all. And I wonder if anyone could speak to the question of how the transmitter levels change in the penis as a function of testosterone. That would perhaps help to reduce the number of hypothetical transmitters that might be involved in erection, because one can assume

that the ones that are testosterone dependent, although they may contribute to erection, may not be necessary for erection.

K.E. Andersson

Recently it was shown that testosterone influences the nitrergic innervation peripherally, so in castration there will be a rather slow disappearance of nitrergic nerves and if you give testosterone supplement, you will restore the number of nerves. I do not think it has been directly shown that castration, for example, which is the way one studies testosterone deprivation in most cases, actually affects the penile erectile tissue or reduces the number of potential transmitters, because response to noradrenaline is still there after castration. But this is an animal study and not a human study. The only thing we found when we studied the effects of the electrical stimulation of penile erectile tissue after castration of rabbits was actually an increase in electrically-induced relaxations and later it was shown that it was dependent on decreased release of noradrenaline.

J. Bancroft

Which raises again the question of whether testosterone is influencing the general variability of noradrenaline so that it might act both in an inhibitory and excitatory way.

M. Baum

Some data recently published (Mills et al, Biol. Reprod. 46: 342, 1992) suggest that testosterone can modulate the ability of N-nitro-L-arginine to disrupt electrically-induced erectile function in rats.

B.D. Sachs

Yes, and I do not know how to interpret those studies in the context of copulatory behaviour. There are unquestionably a large number of androgen-sensitive changes in the penis, in the peripheral nerves going to the penis and in the spinal cord, and those may be important in post-pubertal mammals. However, at least in rats, postcastrational erection is vigorous in the context of copulation 8 to 10 weeks after

castration, and erection is vigorous in severely hypogonadal men in response to erotic videos and is also vigorous in pre-pubertal boys during NPT and in response to touch. That suggests to me that a lot of the androgen-sensitive, even perhaps androgen-dependent responses, are important but not necessary for erection. Or, if they are necessary, I think it is important to demonstrate in what ways they are necessary for -rather than correlative with- erection in normally functioning eugonadal adults.