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Mechanisms of Penile Erection and Basis for Pharmacological Treatment of Erectile Dysfunction

K.-E. Andersson

Wake Forest Institute for Regenerative Medicine, Wake Forest University School of Medicine, Winston Salem, North Carolina

Address correspondence to: Dr. K.-E. Andersson, Wake Forest Institute for Regenerative Medicine, Wake Forest University School of Medicine, Medical Center Boulevard, Winston Salem, NC 27157. E-mail: keanders@wfubmc.edu
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***Abstract*——Erection is basically a spinal reflex that can be initiated by recruitment of penile afferents, both autonomic and somatic, and supraspinal influences from visual, olfactory, and imaginary stimuli. Several central transmitters are involved in the erectile control. Dopamine, acetylcholine, nitric oxide (NO), and peptides, such as oxytocin and adrenocorticotropin/α-melanocyte-stimulating hormone, have a facilitatory role, whereas serotonin may be either facilitatory or inhibitory, and enkephalins are inhibitory. The balance between contractant and relaxant factors controls the degree of contraction of the smooth muscle of the corpora cavernosa (CC) and determines the functional state of the penis. Noradrenaline contracts both CC and penile vessels via stimulation of α_1-adrenoceptors. Neurogenic NO is considered the most important factor for relaxation of penile vessels and CC. The role of other mediators, released from nerves or endothelium, has not been definitely established. Erectile dysfunction (ED), defined as the "inability to achieve or maintain an erection adequate for sexual satisfaction," may have multiple causes and can be classified as psychogenic, vasculogenic or organic, neurologic, and endocrinologic. Many patients with ED respond well to the pharmacological treatments that are currently available, but there are still groups of patients in whom the response is unsatisfactory. The drugs used are able to substitute, partially or completely, the malfunctioning endogenous mechanisms that control penile erection. Most drugs have a direct action on penile tissue facilitating penile smooth muscle relaxation, including oral phosphodiesterase inhibitors and intracavernosal injections of prostaglandin E_1. Irrespective of the underlying cause, these drugs are effective in the majority of cases. Drugs with a central site of action have so far not been very successful. There is a need for therapeutic alternatives. This requires identification of new therapeutic targets and design of new approaches. Research in the field is expanding, and several promising new targets for future drugs have been identified.**

I. Introduction

Penile erection is the end result of a complex neurovascular process in which nerves, endothelium of sinusoids and blood vessels, and smooth muscle cells in the target organ are involved. Erection is basically mediated by a spinal reflex, which, depending on the context in which it occurs, involves different central and peripheral neural and/or humoral mechanisms. In the CNS,[1] there is a processing and integration of tactile, olfactory, auditory and mental stimuli (Fig. 1). Many central nervous and peripheral transmitters and transmitter systems participate in the process. The different steps of neurotransmission, impulse propagation, and intracellular transduction of neural signals in penile smooth muscles are still only partly known. However, it is well established that the balance between contractant and relaxant factors controls the degree of tone of the penile vasculature and of the smooth muscle of the corpora cavernosa (CC) and determines the functional state of the penis: detumescence and flaccidity, tumescence and erection.

Several pharmacological, physiological, and clinical aspects of erectile function and dysfunction have been reviewed previously (e.g., Andersson and Wagner, 1995; Argiolas and Melis, 1995, 2004, 2005; Giuliano and Rampin, 2000, 2004; Andersson, 2001), but the field expands continuously and has been subject to several recent reviews (Baskerville and Douglas, 2008; Burnett et al., 2010; Gratzke et al., 2010a; Melis and Argiolas, 2011). The present review is an attempt to update the rapidly expanding information on some of the transmit-

[1]Abbreviations: α-MSH, α-melanocyte-stimulating hormone; ABT-724, 2-[(4-pyridin-2-ylpiperazin-1-yl)methyl]-1*H*-benzimidazole); ACE, angiotensin-converting enzyme; ACh, acetylcholine; AM251, 1-(2,4-dichlorophenyl)-5-(4-iodophenyl)-4-methyl-*N*-(1-piperidyl)pyrazole-3-carboxamide; AM630, 6-iodo-2-methyl-1-[2-(4-morpholinyl)ethyl]-1*H*-indol-3-yl-(4-methoxyphenyl)methanone; AMPA, amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid; Ang II, angiotensin II; ANP, atrial natriuretic peptide; AR, adrenergic receptor; ARB, angiotensin receptor blocker; BNP, brain natriuretic peptide; cAK, protein kinase A; CB, cannabinoid; CC, corpora cavernosa; cGK, cGMP-dependent protein kinase; CGRP, calcitonin gene-related peptide; CNP, C-type natriuretic peptide; CNS, central nervous system; CV, cardiovascular; ED, erectile dysfunction; eNOS, endothelial nitric-oxide synthase; ET, endothelin; GC, guanylyl cyclase; HO, heme oxygenase; 5-HT, 5-hydroxytryptamine; ICP, intracavernosal pressure; IPA, internal pudendal artery; IP_3, inositol 1,4,5-trisphosphate; L-NAME, N^G-nitro-L-arginine methyl ester; MC, melanocortin; *m*-CCP, *m*-chlorophenylpiperazine; MK-801, dizocilpine maleate; MLC, regulatory light-chain subunits of myosin; MLCK, myosin light-chain kinase; MPOA, medial preoptic area; MT-II, melanotan-II; NA, noradrenaline; NANC, nonadrenergic, noncholinergic; NMDA, *N*-methyl-D-aspartic acid; nNOS, neuronal nitric-oxide synthase; NOS, nitric-oxide synthase; PACAP, pituitary adenylate cyclase-activating polypeptide; PDE, phosphodiesterase; PG, prostaglandin; pGC, particulate GC; PKC, protein kinase C; PKG, protein kinase G; PVN, paraventricular nucleus; R 141716A, *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboximide hydrochloride; RAS, renin-angiotensin system; ROS, reactive oxygen species; sGC, soluble guanylyl cyclase; SHU-9119, Ac-Nle-cyclo(Asp-His-D-2-Nal-Arg-Trp-Lys)-NH; SIN-1, 3-morpholinosydnonimin hydrochloride (linsidomine); SM, smooth muscle; SNP, sodium nitroprusside; TNF, tumor necrosis factor; UK-14,304, 5-bromo-*N*-(4,5-dihydro-1*H*-imidazol-2-yl)-6-quinoxalinamine; VIP, vasoactive intestinal peptide; VPAC, VIP receptor; Y-27,632, *trans*-4-[(1*R*)-1-aminoethyl]-*N*-4-pyridinylcyclohexanecarboxamide dihydrochloride; YC-1, 3-(5′-hydroxymethyl-2′-furyl)-1-benzylindazole.

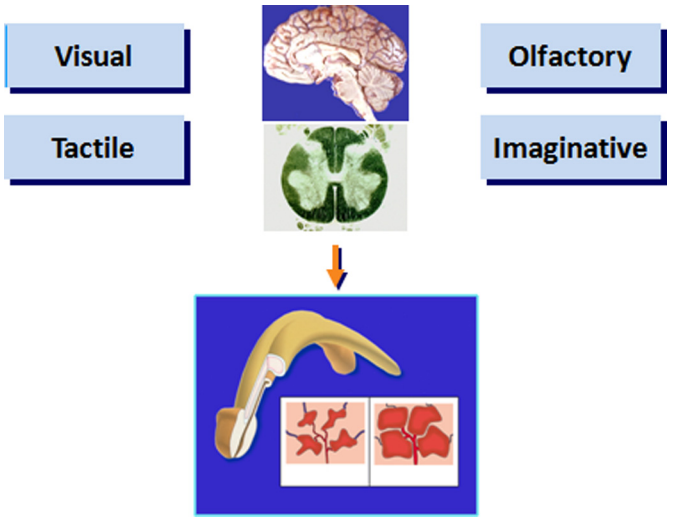

FIG. 1. Penile erection is basically a spinal reflex that can be initiated by stimuli from the periphery and from the central nervous system.

ters/modulators believed to be involved in the control of erectile mechanisms centrally and peripherally and that are the basis for the currently used treatments of erectile dysfunction (ED). The review is by no means complete; keeping some of the perspectives from previous reviews in the field (Andersson, 1993, 2001; Andersson and Wagner, 1995), focus has been given to contributions from the last decade.

II. Central Regulation

Some of the anatomical areas of the brain that relate to sexual function have been defined. Evidence from animal studies indicates that the central supraspinal systems controlling sexual arousal are localized predominantly in the limbic system (e.g., olfactory nuclei, medial preoptic area, nucleus accumbens, amygdala, and hippocampus) and hypothalamus (paraventricular and ventromedial nuclei). In particular, medial amygdala, medial preoptic area (MPOA), paraventricular nucleus (PVN), the periaqueductal gray, and ventral tegmentum are recognized as key structures in the central control of the male sexual response (Andersson and Wagner, 1995; Giuliano and Rampin, 2000a,b; Argiolas and Melis, 2005; Hull and Dominguez, 2007; Melis and Argiolas, 2011). In rats, electrical stimulation of the MPOA, the PVN, or the hippocampal formation can elicit an erectile response. There seems to be a spinal network consisting of primary afferents from the genitals, spinal interneurons, and sympathetic, parasympathetic, and somatic nuclei. This network seems capable of integrating information from the periphery and eliciting reflexive erections and also seems to be the recipient of supraspinal information (Giuliano and Rampin, 2000a,b). In humans, the physiologic link between these brain regions and male sexual arousal associated with the remote sexual response has been little studied and remains an issue of debate. Studies using functional magnetic resonance imaging or positron emission tomography have elucidated patterns of brain activation correlated with the different phases of sexual response. Activation maps highlighted a complex neural circuit involved in sexual arousal. Of this circuit, only a few areas (anterior cingulate, insula, amygdala, hypothalamus, and secondary somatosensory cortices) were specifically correlated with penile erection (Ferretti et al., 2005; Miyagawa et al., 2007). Further research in these fields is needed and may be rewarding.

III. Central Mediators

The central nervous regulation of erectile function involves both spinal and supraspinal pathways and mechanisms. Not unexpectedly, the central neurotransmission of penile erection is complex and only partly known. However, progress continues to be made to identify effectors involved in this function, and the field has recently been reviewed (Melis and Argiolas, 2011). Much of the knowledge gained in this area of research relates to morphological and pharmacological studies in experimental animal models (e.g., rodents, rabbits, primates). In these models, neurochemical perturbations can be performed and responses monitored in a reasonably meaningful way. Results of such investigations must be interpreted with caution, because they encompass a wide range of types and modes of elicitation of sexual function (Sachs, 2000). Species differences, drug-dependent effects, and multiple drug sites of action must also be considered (McKenna, 1999; Giuliano and Rampin, 2000a,b; Steers, 2000).

Among central neurotransmitters and neuropeptides that control penile erection, the best known are serotonin, dopamine, oxytocin, excitatory amino acids, NO, adrenocorticotropin/α-melanocyte stimulating hormone (α-MSH), and opioid peptides. They can facilitate or inhibit penile erection by acting in several brain areas (i.e., the MPOA, the PVN, the ventral tegmental area, the hippocampus, the amygdala, the bed nucleus of the stria terminalis, the nucleus accumbens, the medulla oblongata and the spinal cord) (Melis and Argiolas, 2011). The PVN seems to have a central role and NO and oxytocin seem to be main players in the mediation of the effect (Fig. 2). Androgens also have an important role; e.g., lack of testosterone may reduce or abolish the effects of many of the erection-mediating transmitters.

A. 5-Hydroxytryptamine

5-Hydroxytryptamine (5-HT; serotonin) has been implicated in the supraspinal as well as the spinal pharmacology of erectile function in both animals and humans. 5-HT is considered to exert a general inhibitory effect on male sexual behavior and involves both sympathetic, parasympathetic, and somatic outflow mechanisms (Bitran and Hull, 1987; Hull et al., 2004). 5-HT-positive nerve terminals are present throughout the

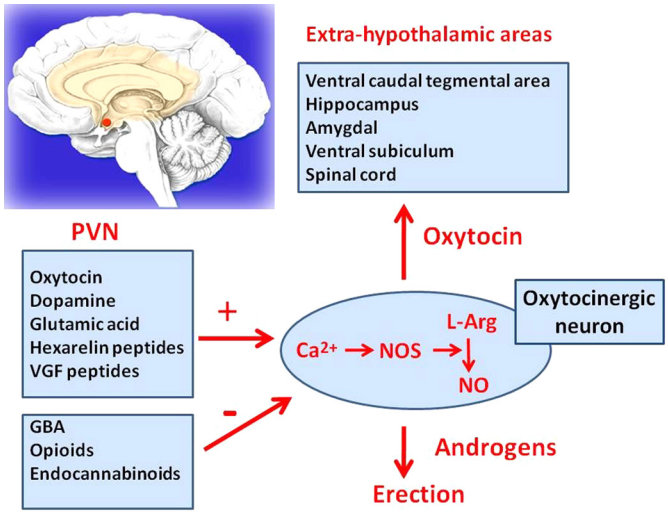

FIG. 2. The PVN of the hypothalamus, oxytocin, and neurons containing oxytocin play important roles in the central control of erection. Oxytocinergic neurons originating in the PVN project to extrahypothalamic brain areas (e.g., the ventral caudal tegmental area, the hippocampus, the amygdala, and the spinal cord). These neurons are activated by oxytocin itself, dopamine, excitatory amino acids, VGF-derived peptides, and hexarelin analog peptides and inhibited by stimulation of GABA, opioids, and cannabinoids. The activation of oxytocinergic neurons follows the activation of NOS present in these neurons. The NO-mediated activation of oxytocinergic neurons is apparently not related to the stimulation of guanylyl cyclase; it causes release of oxytocin in the spinal cord and in extrahypothalamic brain areas. In the mediation of erection, androgens play an important role. [Modified from Melis MR and Argiolas A (2011) Central control of penile erection: a re-visitation of the role of oxytocin and its interaction with dopamine and glutamic acid in male rats. *Neurosci Biobehav Rev* **35:**939–955. Copyright © 2011 Elsevier. Used with permission.].

central nervous system, and 5-HT-containing neurons can be found in the medullary raphe nuclei and ventral medullary reticular formation, including the rostral nucleus paragigantocellularis, as well as the lumbosacral spinal cord in association with mainly somatic and autonomic outflow projections to the pelvis (Andersson, 2001). A decreased amount of 5-HT in these structures, induced experimentally by the inhibition of serotonin synthesis (parachlorophenylalanine), destruction of 5-HT-containing axons (5,7-dihydroxytryptamine), or electrolytic destruction of the dorsal raphe nucleus, enhances sexual activity (McIntosh and Barfield, 1984; Kondo et al., 1993). Conversely, sexual activity is attenuated after the intracerebroventricular or intrathecal administration of 5-HT and drugs that increase central release or synthesis of amine (Ahlenius et al., 1981; Svensson and Hansen, 1984; Szele et al., 1988).

The 5-HT pathways may be inhibitory or facilitatory depending upon the action of the amine at various subtypes of 5-HT receptors located at different sites in the central nervous system (de Groat and Booth, 1993). The effects also seem to be species-specific (Paredes et al., 2000). Intrathecal injection of 5-HT in the spinalized anesthetized male rat blocked the appearance of the coitus reflex, suggesting that endogenous 5-HT may act in the descending input to the lumbar spinal cord that inhibits sexual reflexes (Marson and McKenna, 1992). A similar procedure in other experiments also inhibited ejaculation as well as penile intromission in rats, suggesting an alternative role of 5-HT in the transmission of sensory feedback information necessary for sexual responses (Svensson and Hansen, 1984).

Many 5-HT receptor subtypes have been identified, and the receptors use different effector systems in different cells, which may explain the conflicting reports on the effects of 5-HT agonists and antagonists on sexual functions. For example, agonists may either enhance or depress sexual function. 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A}, and 5-HT_{2C} receptor subtypes have been found at different levels of the spinal cord (Marlier et al., 1991; Thor et al., 1993; Ridet et al., 1994). In accordance with the selective use of 5-HT receptor agonists and antagonists, components of male copulatory behavior were found to be displayed variably. For example, 5-HT_{1A} receptor activation may have contrasting effects on sexual function, depending on the dose of administration and location of the receptor in the brain (Ahlenius and Larsson, 1997; Rehman et al., 1999). Bancila et al. (1999), using immunohistochemistry, suggested on the basis of their findings that the supraspinal serotonergic control of erection at the lumbosacral level seemed to be strongly associated with activation of 5-HT_{2C} receptors. 1-(3-Chlorophenyl)-piperazine, a trazodone metabolite, and *N*-trifluoromethylphenyl-piperazine, are considered partial agonists at 5-HT_{2C} receptors and usually display 5-HT_{2A} receptor antagonistic actions (Barnes and Sharp, 1999). They both induce erection in rodents, but they also significantly inhibit ejaculation and sexual behavior (Andersson, 2001).

In rats, 5-HT, dopamine, oxytocin, and melanocortin pathways are known to be involved in the induction of penile erections. A dopamine–oxytocin–5-HT link has been suggested to be important, but the 5-HT receptor subtype that mediates the dopamine–oxytocin–5-HT action and the relationship between the dopamine–oxytocin–5-HT and melanocortin pathways have not been fully elucidated. Kimura et al. (2008) suggested that 5-HT_{2C} receptors in the lumbosacral spinal sites mediate not only dopamine–oxytocin–5-HT action, but also melanocortin effects on penile erections and that the 5-HT pathway is located downstream from the melanocortin as well as the dopamine–oxytocin pathways.

Drugs that act through 5-HT mechanisms may affect sexual behavior. Thus, melatonin, which increases all aspects of sexual activity in rats, possesses 5-HT_{2A}-antagonistic properties (Drago et al., 1999). Evidence for a facilitatory role of melatonin in sexual behavior has been presented, suggesting that its mechanism of action may involve the 5-HT_{2A} receptor (Brotto and Gorzalka, 2000). Agomelatine, an antidepressant with melatonin agonist and 5-HT_{2C}-antagonist properties, was found to antagonize the penile erections induced by the stimulation of 5-HT_{2C} receptors in Wistar rats (Chagraoui et al., 2003).

Few drugs with a direct action on 5-HT mechanisms are in clinical use to promote erection (e.g., trazodone). However, the potential of such drugs for treatment of ED is promising. Considering the negative effects of selective serotonin-reuptake inhibitors and serotonin- and NA-reuptake inhibitors on sexual function (Corona et al., 2009), further studies on the influence of 5-HT on erectile mechanisms are desirable.

B. Dopamine

Dopamine is the main catecholamine in the CNS and is involved in a variety of physiological functions, including sexual behavior. Dopamine has facilitative effects on sexual motivation, copulatory proficiency, and genital reflexes (Hull et al., 2004). Dopaminergic neurons comprise an incertohypothalamic system with projections to the MPOA and PVN (Björklund et al., 1975). In the MPOA, dopamine controls genital reflexes, copulatory patterns, and specifically, sexual motivation (Hull et al., 2004). Dopaminergic neurons have also been identified traveling from the caudal hypothalamus within the diencephalospinal dopamine pathway to innervate the lumbosacral spinal cord (Skagerberg et al., 1982; Skagerberg and Lindvall, 1985). Dopamine may thus be expected to participate in the central regulation of both the autonomic and somatic components of the penile reflexes, and this has been confirmed by the effects of apomorphine. Dopamine receptors in mammalian tissues have been classified as D_1-like (D_1 and D_5) and D_2-like (D_2, D3, and D_4) based on their binding properties and their ability to activate or inhibit forskolin-induced adenylyl cyclase activity (Beaulieu and Gainetdinov, 2011). In the CNS, both the families have been associated with erectile functions.

An important finding was the discovery of the expression of all dopamine receptors of the D_2 receptor family (D_2, D_3 and D_4) in the cell bodies of oxytocinergic neurons in the PVN, the SON, and the MPOA (Baskerville and Douglas, 2008; Baskerville et al., 2009), which provides strong neuroanatomical support to the hypothesis that dopamine and dopamine receptor agonists may activate directly oxytocinergic neurons involved in erectile function.

The participation of dopamine in sexual function, including erection, is further supported by studies demonstrating that several dopamine receptor agonists, such as apomorphine, quinpirole, quinelorane, and (−)-3-(3-hydroxyphenyl)-*N*-*n*-propylpiperidine, induce penile erection after systemic administration in mammals (Melis and Argiolas, 1995). These drugs are known to induce nausea and emesis, which limits their clinical usefulness. In rats and rabbits, the proerectile effect of apomorphine exhibits a characteristic inverted-U response.

Erection after dopamine stimulation involves oxytocinergic neurotransmission (Baskerville et al., 2009; Melis and Argiolas, 2011). Dopaminergic neurons impinge on oxytocinergic cell bodies in the PVN (Buijs, 1978; Lindvall et al., 1984), and apomorphine-induced penile erection is prevented dose-dependently by oxytocin receptor antagonists or by electrolytic lesions of the PVN that deplete central oxytocin content. Conversely, injection of oxytocin into the PVN induced erections that were not attenuated by dopamine receptor blockade, suggesting that dopaminergic neurons activate oxytocinergic neurons in the PVN and that released oxytocin then accounts for the erectile response (Baskerville et al., 2009: Melis and Argiolas, 2011).

It has been suggested that in the PVN, dopamine-induced oxytocinergic activation may involve a calcium-dependent nitric oxide (NO) pathway rather than the classic cAMP pathway. Thus, intra-PVN injection of ω-conotoxin-GVIA, a selective antagonist of N-type calcium channels, inhibited apomorphine- and oxytocin-induced penile erection. Blockade of the N-type calcium channels also attenuated the increase in nitrite and nitrate concentrations (indicators of NO activity) during penile erection (Succu et al., 1998). Neuronal NOS is abundantly expressed in oxytocinergic neurons (Ferrini et al., 2001; Xiao et al., 2005), and centrally administered NOS inhibitors were shown to prevent dopamine agonist and oxytocin-induced penile erection.

Testosterone increases NOS in the MPOA. NO in turn increases basal and female-stimulated dopamine release, which facilitates copulation and genital reflexes. Dopamine-receptor agonist-induced erections were abolished by castration in rodents, and testosterone replacement restored erectile function (Hull et al., 2004).

It has been reported that the proerectile effect of apomorphine is mediated via the specific D_2 receptor subtype; however, studies with selective dopamine agonists did not confirm this hypothesis (Hsieh et al., 2004). The dopamine D_4 receptor is expressed in brain areas such as the prefrontal cortex, hippocampus, amygdala, and hypothalamus, which are known to control sexual function in mammals (Primus et al., 1997). ABT-724 (2-[(4-pyridin-2-ylpiperazin-1-yl)methyl]-1*H*-benzimidazole) is a selective dopamine D_4 receptor agonist that activates human dopamine D_4 receptors with no effect on dopamine D_1, D_2, D_3, or D_5 receptors (Brioni et al., 2004). The drug dose-dependently facilitated penile erection when given subcutaneously to conscious rats, an effect that was blocked by haloperidol and clozapine (acting centrally), but not by domperidone (acting peripherally). A proerectile effect was observed after intracerebroventricular but not intrathecal administration, suggesting a supraspinal site of action. In the presence of sildenafil, a potentiation of the proerectile effect of ABT-724 was observed in conscious rats. ABT-724 was evaluated in conscious male ferrets, a preclinical model to determine the emetic potential of drugs. ABT-724 did not cause emesis or nauseogenic behavior, despite its ability to activate ferret D_4 receptors. The ability of ABT-724 to facilitate penile erection together with the favorable

side-effect profile suggested that ABT-724 could be useful for the treatment of erectile dysfunction (Brioni et al., 2004). For unknown reasons, the drug does not seem to have been further developed, and no experiences from its use in humans have been published.

C. Oxytocin

In the PVN of the hypothalamus, pharmacological, immunocytochemical, and electrophysiological studies have identified a group of oxytocinergic neurons projecting to extrahypothalamic brain areas and the spinal cord, which influence erectile function. When activated by, for example, dopamine, excitatory amino acids, by oxytocin itself, and by hexarelin analog peptides, these neurons will produce penile erection (Argiolas and Melis, 2004; Baskerville and Douglas, 2008; Melis and Argiolas, 2011). Oxytocin facilitates erectile function and male sexual behavior in, for example, mice, rats, rabbits, and monkeys. This may occur also in humans, because plasma oxytocin is increased by sexual stimuli, especially at ejaculation (Carmichael et al., 1987; Murphy et al., 1987). Oxytocin induces penile erection not only when injected into the lateral cerebral ventricle and the PVN but also in other extrahypothalamic brain regions, such as the ventral tegmental area (Melis et al., 2007, 2009; Succu et al., 2008), the ventral subiculum of the hippocampus, and the posterior nucleus of the amygdala (Melis et al., 2009, 2010), which are important constituents of the limbic system and are thought to play a key role in motivation and reward processes. The erectile response was blocked by oxytocin antagonists and by electrolytic lesion of the PVN. The oxytocin-induced erections were also abolished by castration, and testosterone replacement restored erectile function (Argiolas and Melis, 2004; Baskerville and Douglas, 2008).

Oxytocin seems to exert an autoactivation mechanism involving stimulation of oxytocinergic receptors located on the cell bodies of the same oxytocinergic neurons in the PVN (Argiolas and Melis, 2004). In support of this view, immunoreactive cell bodies of oxytocinergic synapses have been found to impinge upon the cell bodies of oxytocinergic neurons in both hypothalamic supraoptic and PVN nuclei (Theodosis, 1985). Several central neurotransmitters may also converge upon the oxytocinergic system as activators (e.g., dopamine) or inhibitors (e.g., opioid peptides) of its transmission. The activation of these oxytocinergic neurons controlling erectile function and sexual behavior is mediated by the activation of NOS.

Stimulation of oxytocin receptors is believed to increase Ca^{2+} influx inside the cell bodies of oxytocinergic neurons. In line with this hypothesis, Ca^{2+} channel blockers reduce oxytocin-induced erection, particularly ω-conotoxin GVIA, a selective blocker of the N-type voltage-dependent Ca^{2+} channels. Apparently, the increase in Ca^{2+} influx causes activation of neuronal NOS (nNOS), leading to an increase in NO production in the PVN. NO in turn activates oxytocinergic neurons to release oxytocin in extrahypothalamic brain areas and the spinal cord to induce penile erection.

The PVN is rich in NOS present in the cell bodies of oxytocinergic neurons projecting to extrahypothalamic brain areas. Oxytocin-induced penile erection is reduced by NOS inhibitors given into the PVN with a potency parallel to that exerted by these compounds in inhibiting NOS (Melis et al., 1994c; Melis and Argiolas, 1997). NO donors injected into the PVN induce penile erection episodes that are reduced by oxytocin receptor antagonists given into the lateral ventricles. Microdialysis studies have shown that an increase in NO production occurs in the PVN concomitantly to penile erection in rats treated with oxytocin (Melis et al., 1997c), and this increase is reduced by NOS inhibitors given into the PVN at doses that reduce the number of penile erection episodes induced by the peptide. The mechanisms by which NO activates PVN oxytocinergic neurons controlling erectile function are unknown. Guanylyl cyclase is apparently not involved at the level of the PVN (Melis and Argiolas, 2011).

The spinal cord contains oxytocinergic fibers and receptors (Freund-Mercier et al., 1987; Uhl-Bronner et al., 2005), and intrathecal oxytocin induces penile erection (Tang et al., 1998; Véronneau-Longueville et al., 1999; Giuliano and Rampin, 2000a; Giuliano et al., 2001). These oxytocinergic fibers originate in the PVN and contribute to descending pathways controlling spinal autonomic neurons mediating penile erection. They make synaptic contacts in the dorsal horn preganglionic sympathetic and parasympathetic cell columns in the thoracolumbar and lumbosacral tract with spinal neurons innervating CC (Marson and McKenna, 1996; Giuliano and Rampin, 2000a; Giuliano et al., 2001). Thus, oxytocin, released during physiological activation of the PVN, is a potent activator of spinal proerectile neurons projecting to the CC.

Despite its central role for erection in rodents, it is still not known whether oxytocin has the same importance in humans. After systemic administration, oxytocin will most probably not reach concentrations in the brain that can affect erectile mechanisms. An oxytocin analog (nonpeptide) able to penetrate the blood-brain barrier would be of interest but apparently remains to be developed.

D. Noradrenaline

A small number of nuclei, including the locus ceruleus, send noradrenergic fibers to the forebrain and spinal cord, including those areas controlling penile erection. In general, evidence for noradrenergic mechanisms involved in the supraspinal mediation of penile erection is sparse. Noradrenergic neurons from the A5 region and from the locus ceruleus project to the nuclei in the spinal cord involved in erection (Giuliano and Rampin, 2000b). Available data suggest that increased

central noradrenergic activity stimulates sexual function, whereas decreased activity inhibits it (Bitran and Hull, 1987). Insights have almost exclusively been drawn from experimental work involving the administration of agents that interact through α-adrenoceptor (AR) pathways. Male sexual behavior was suppressed in rats given the α_2-AR agonist clonidine by direct injection into the MPOA (Clark, 1988). The suppression was inhibited by pretreatment with selective α_2-AR antagonists (Clark et al., 1985), consistent with established facilitatory effects of these agents on erectile responses in rats (Clark et al., 1985). However, although several α_2-AR antagonists, most notably yohimbine, have been shown to increase sexual responses in rats, the relatively poor therapeutic efficacy of yohimbine in men with ED (see section VIII.C) casts doubt on the significance of central noradrenergic mechanisms in erectile function.

E. Excitatory Amino Acids

N-methyl-D-aspartic acid (NMDA), a selective agonist of the NMDA receptor subtype, but not amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid (AMPA), a selective agonist of the AMPA receptor subtype, or *trans*(1)-amino-1,3-cyclopentane dicarboxylic acid, a selective agonist of the metabotropic receptor subtype, was found capable of inducing penile erection when injected into the PVN of freely moving rats (Melis et al., 1994b; Argiolas and Melis, 2005). The NMDA effect was prevented by NMDA receptor antagonists such as dizocilpine maleate (MK-801) and by intracerebroventricular administration of an oxytocin antagonist (Melis et al., 1994b). Glutamate is released in the MPOA of male rats during copulation (Dominguez, 2009), and microinjections of L-glutamate into the MPOA elicited an increase in intracavernosal pressure (Giuliano et al., 1996). The NOS signal transduction pathway is considered to mediate the effect of NMDA, because the intracerebroventricular administration of NOS inhibitors into the PVN blocked the NMDA effect (Argiolas, 1994; Melis et al., 1994a). Further support was provided by findings that NMDA injected into the PVN also leads to an increased concentration of NO metabolites in this region (Melis et al., 1997b). It is likely that NMDA receptors mediating penile erection are located on the cell bodies of oxytocinergic neurons, because excitatory amino acid-containing nerve endings impinge on oxytocinergic cell bodies in the PVN (van den Pol, 1991). The proerectile effect of NMDA is thus mediated by the activation of oxytocinergic neurotransmission and abolished by a selective oxytocin receptor antagonist given into the lateral ventricles but not into the PVN (Argiolas, 1999). The NMDA receptor-mediated activation of NOS may be secondary to an increased Ca^{2+} influx in oxytocinergic cell bodies through the Ca^{2+}-channel-coupled NMDA receptors. NO in turn activates oxytocinergic transmission. However, the ineffectiveness of ω-conotoxin injected into the PVN in blocking erections induced by NMDA, also injected in this nucleus, indicates that ω-conotoxin-sensitive N-type calcium channels are not responsible for this mediation (Succu et al., 1998). The origin of glutamatergic projections that activate paraventricular oxytocinergic neurons mediating penile erection is unknown.

The spinal cord of the rat contains receptors for both NMDA and AMPA. In anesthetized rats, the combined administration of the glutamatergic agonists of NMDA and AMPA receptors elicited increases in intracavernosal pressure in the absence of stimulation of the dorsal penile nerve (Rampin et al., 2004). It was hypothesized that glutamate, released on stimulation of the genitals and acting at AMPA and NMDA receptors, is a potent activator of the spinal proerectile network. It remains to be established whether drugs influencing glutaminergic mechanisms will be useful for ED treatment in humans.

F. GABA

Studies on the role of GABA in penile erection indicate that this neurotransmitter may function as an inhibitory modulator in the autonomic and somatic reflex pathways involved in penile erection (de Groat and Booth, 1993). In male rats, high concentrations of GABA have been measured in the medial preoptic area of the hypothalamus (Elekes et al., 1986), and GABAergic fibers and receptor sites have been localized to the sacral parasympathetic nucleus and bulbocavernosus motor nucleus (Bowery et al., 1987; Magoul et al., 1987). Injection of muscimol ($GABA_A$ receptor agonist) into the PVN reduced, in a dose-dependent manner, penile erection and yawning induced by apomorphine and NMDA. The reduction of penile erection (and yawning) was parallel to a reduction of the concomitant NO^{2+} and NO^{3+} increase. In contrast, baclofen ($GABA_B$ receptor agonist) was ineffective (Melis and Argiolas, 2002). The injection of $GABA_A$ receptor agonists into the MPOA decreased copulatory behavior of male rats (Fernández-Guasti et al., 1986), whereas the injection of $GABA_A$ receptor antagonists into this region increased such behavior (Fernández-Guasti et al., 1985). Systemic administration or intrathecal injection at the lumbosacral level of the $GABA_B$ receptor agonist baclofen decreased the frequency of erections in rats (Bitran and Hull, 1987). Activation of $GABA_A$ receptors in the PVN reduced apomorphine-, NMDA-, and oxytocin-induced penile erection and yawning in male rats (Melis and Argiolas, 2002). Such activation also reduced penile erection induced by hexarelin analog peptides by diminishing the increase in NO activity that simultaneously occurs in this hypothalamic nucleus (Succu et al., 2003).

Stimulation of $GABA_A$ and $GABA_B$ receptors may produce different (e.g., inhibitory or excitatory) effects on yawning and penile erection, depending on the brain area in which they act. $GABA_A$ receptors in the PVN inhibit yawning and penile erection occurring in different contexts and show that this inhibition is mediated by a decrease of the NOS activation that occurs during

these behavioral responses in this hypothalamic nucleus. Despite the physiological importance of GABA for erection, no drugs interfering with GABA mechanisms seem to have been developed with the aim to treat ED.

G. Adrenocorticotropin and Related Peptides

The melanocortins have a wide variety of effects in the brain (Bertolini et al., 2009). Proteolytic cleavage of the precursor pro-opiomelanocortin gives rise to several peptides, including adrenocorticotropin and α-MSH, both of which have been associated with erectile responses. After intracerebroventricular or hypothalamic periventricular injection in various animal models, they induce penile erection and ejaculation, grooming, stretching, and yawning (Wessells et al., 2005; King et al., 2007). These effects were shown to be androgen-dependent, because they were abolished by castration and could be fully restored by treating castrated animals with testosterone (Bertolini et al., 1975). It is noteworthy that adrenocorticotropin and the adrenocorticotropin-like peptides did not enhance social interaction in rats, because they did not seek to copulate with partners during periods of sexual stimulation (Bertolini and Gessa, 1981).

It is now clear that most, if not all, of the effects of the α-MSH/adrenocorticotropin peptides are mediated via specific subtypes of melanocortin (MC) receptors. Of the five cloned melanocortin receptor subtypes, only the MC_3 and MC_4 receptors have been identified in CNS regions associated with activation of penile erection (Wikberg et al., 2000), particularly the PVN of the hypothalamus. α-MSH/adrenocorticotropin peptides seem to act in the hypothalamic periventricular region, and grooming, stretching, and yawning, but not penile erection, seem to be mediated by MC_4 receptors (Vergoni et al., 1998; Argiolas et al., 2000). It is noteworthy that the MC_3 receptor showed a high density in the hypothalamus and limbic systems (Wikberg, 1999), regions known to be important for erectile functions. However, there are conflicting data as to which receptor mediates erection. The MC_4 receptor is emerging as the principle effector of MC-induced erection (Martin and MacIntyre, 2004), but the role of the MC_3 receptor is poorly understood.

Calcium channels seem to mediate the effects of α-MSH/adrenocorticotropin peptides, because intracerebroventricular injection of the N-type calcium channel blocker ω-conotoxin prevents the actions of adrenocorticotropin (Argiolas et al., 1990). Intracerebroventricular injection of L-NAME significantly inhibited adrenocorticotropin-induced erections but not stretching and grooming. Both lesions of the PVN (Argiolas et al., 1987) and injections of ω-conotoxin into this nucleus (Argiolas et al., 1990) failed to alter erection induction by adrenocorticotropin. This observation, combined with evidence that excitatory amino acids do not affect adrenocorticotropin effects (Melis et al., 1992a), suggest that the hypothalamic site, or the mechanism of action responsible for adrenocorticotropin induction of erection, is different from that involving dopamine or oxytocin action in the PVN. However, NO seems to be involved in the adrenocorticotropin effects (Poggioli et al., 1995). Magnocellular oxytocin neurons were found to be involved in the central regulation of male sexual behavior, and some of the central effects of α-MSH are likely to be mediated by magnocellular oxytocin neurons (Caquineau et al., 2006).

Proerectile functions of spinal melanocortin receptors have been proposed, and spinal MC_4 receptor mRNA expression has been demonstrated (Van der Ploeg et al., 2002). Intrathecal injection of the MC receptor agonist melanotan-II (MT-II) to the lumbar spinal cord dose-dependently increased spontaneous erections in male rats (Wessells et al., 2003) This effect was abolished by intrathecal coadministration of the MC receptor antagonist Ac-Nle-cyclo(Asp-His-D-2-Nal-Arg-Trp-Lys)-NH_2 (SHU-9119). When SHU-9119 was given intracerebroventricularly, it did not block MT-II spinally induced erections. These results suggest that MC receptor agonists act on independent spinal loci for initiation of erection.

H. Opioid Peptides

Endogenous opioid peptides have long been assumed to be involved in the regulation of male sexual responses, because sexual dysfunction has been observed clinically in men with long-term opiate use (Cushman, 1972; Crowley and Simpson, 1978). Men on methadone or buprenorphine maintenance treatment were shown to have a high prevalence of ED, related to hypogonadism and depression (Hallinan et al., 2008).

Copulatory behavior in male rats is depressed experimentally with the systemic administration of morphine or other opioids (McIntosh et al., 1980; Pfaus and Gorzalka, 1987). β-Endorphin injection into the cerebral ventricles or MPOA of male rats attenuates copulatory behavior (McIntosh et al., 1980; Hughes et al., 1987). Morphine, injected systemically or into the PVN of male rats, prevents penile erection induced by intracerebroventricular administration of oxytocin or subcutaneous dopamine (Melis et al., 1992b) and by NMDA (Melis et al., 1997a) and cannabinoid antagonists (Succu et al., 2006) injected into the PVN. However, similar application of a selective agonist of the κ opioid receptor does not alter apomorphine- or oxytocin-induced erectile responses (Melis et al., 1997d). This evidence and the demonstration that the opiate antagonist naloxone administered systemically abolishes the central morphine preventative effect on erections in rats have supported the belief that μ receptors in the PVN account for the morphine effect (Melis et al., 1997d; Succu et al., 2006). NO metabolite concentrations that are increased in the PVN after apomorphine, oxytocin, or NMDA local administration, were reduced after morphine administration into the PVN, indicating that the morphine effect

depresses a NO-mediated erection induction mechanism at this level (Melis et al., 1997a,d; Succu et al., 2006). Current data support the hypothesis that opioid μ receptor stimulation centrally prevents penile erection by inhibiting mechanisms that converge upon central oxytocinergic neurotransmission.

I. Acetylcholine

The role of acetylcholine (ACh) at central levels in the regulation of penile erection is mostly inferred from limited neuropharmacologic studies involving systemically and/or intracerebrally administered muscarinic agonists and antagonists and from lesioning studies in the brain (Hull et al., 1988a,b; Maeda et al., 1990, 1994a,b). These studies have suggested that cholinergic mechanisms operating seemingly at the hippocampus and MPOA may have a regulatory role in erectile function.

J. Hexarelin Analog Peptides

Hexarelin analog peptides derive from hexarelin, a peptide initially characterized for its ability to release growth hormone in laboratory animals and in humans (Argiolas and Melis, 2005). A few of these peptides were found to be able to induce penile erection when injected into the PVN and, to a lesser extent, when given systemically (Melis et al., 2001; Argiolas and Melis, 2005). Some hexarelin analog peptides injected into the PVN were found to have a potency in inducing penile erection comparable on a molar basis to that of dopamine agonists oxytocin and NMDA (Melis et al., 2000). The available experimental evidence suggests that hexarelin analogs induce penile erection by activating paraventricular oxytocinergic neurons projecting to extrahypothalamic brain areas. Indeed, their proerectile effect is reduced by an oxytocin antagonist given into the lateral ventricles but not into the PVN (Melis et al., 2001). Structure-activity relationship studies suggest that the peptides with proerectile activity induce penile erection by stimulating specific receptors, other than those previously characterized that mediate growth hormone release and feeding behavior (Melis et al., 2000). These receptors are probably located on the cell bodies of oxytocinergic neurons mediating penile erection (Melis et al., 2001). Apparently, the activation of the hexarelin receptors induces penile erection by increasing Ca^{2+} influx into the cell bodies of oxytocinergic neurons, which causes the activation of NOS, as reported for dopamine agonists oxytocin and NMDA. NO in turn activates the oxytocinergic neurons. Accordingly, hexarelin analog peptide-induced penile erection occurs concomitant with an increased NO production in the PVN and can be prevented by the inhibition of paraventricular NOS and by the blockade of N-type voltage-dependent Ca^{2+} channels by conotoxin (Melis et al., 2000), and by oxytocin receptor antagonists given into the lateral ventricles, but not in the PVN (Melis et al., 2001; Argiolas and Melis, 2005). The importance of the hexarelin system for erection in humans is unknown, and whether hexarelin analog peptides can be developed to drugs for ED treatment remains to be established.

K. Cannabinoids

Administration of endogenous and exogenous cannabinoids was shown to be associated with changes in penile erection and modulation of male sexual behavior (Shrenker and Bartke, 1985; Ferrari et al., 2000). The cannabinoid CB1 receptor antagonist *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboximide hydrochloride (SR 141716A) was found to induce erection when injected to the PVN (Melis et al., 2004) and potentiated the penile erection responses to apomorphine in rats (da Silva et al., 2003). The proerectile effect of SR 141716A was reduced by blockade of NMDA receptors and by NOS inhibition but not by blockade of dopamine or oxytocin receptors in the PVN. However, the erection action was blocked if oxytocin receptor antagonists were given into the lateral ventricle (Melis et al., 2004). Cannabinoid CB1 receptors have been demonstrated in the PVN and may influence erectile function and sexual activity, possibly by modulating paraventricular oxytocinergic neurons mediating erectile function (Melis et al., 2004). It cannot be excluded that SR 141716 induced penile erection by a mechanism involving excitatory amino acid neurotransmission causing activation of nNOS in paraventricular oxytocinergic neurons (Melis et al., 2006).

L. Pro-VGF-Derived Peptides

The *vgf* gene encodes for VGF (nonacronymic), a 617-amino acid precursor protein with a tissue-specific pattern of expression limited to neurons in the central and peripheral nervous systems and to specific populations of endocrine cells (Levi et al., 2004). Immunocytochemical studies have revealed numerous VGF-containing neuronal fibers and terminals within the PVN, including its parvocellular components, and many VGF-immunostained neuronal terminals impinged onto parvocellular oxytocinergic neurons (Succu et al., 2004). Some pro-VGF-derived peptides have been shown to induce penile erection when injected into the PVN of male rats. These peptides derive from the proteolytic cleavage of VGF. So far, the effect of five peptides derived from the C-terminal portion of rat pro-VGF have been studied after injection into the PVN (Argiolas and Melis, 2005). VGF588–617-induced penile erection was reduced by L-NAME and by oxytocin receptor antagonist when given into the lateral ventricles but not when injected into the PVN (Succu et al., 2004). It was considered likely that pro-VGF-derived peptides facilitate erectile function by increasing oxytocinergic neurotransmission.

Available data suggest that within the PVN, pro-VGF-derived peptides may be released under physiological circumstances to influence sexual function by activating paraventricular oxytocinergic neurons mediating penile erection. The pro-erectile effect of VGF peptides occurs

concomitantly with an increase in paraventricular NO production, an increase that is reduced by NOS inhibition, as found with other compounds that induce penile erection when injected into the PVN (Succu et al., 2005). It is noteworthy that the absence of pro-VGF protein and its derived peptides, as it occurs in VGF-knockout mice, resulted in dramatically impaired sexual behavior, sexual maturation, and fertility (Salton et al., 2000).

M. Nitric Oxide

The role of NO in the central neuromediation of penile erection was revealed after the observations that the injection of NOS inhibitors intracerebroventricularly or into the PVN prevented penile erectile responses induced in rats by the dopamine agonists oxytocin and adrenocorticotropin, by 5-HT_{2C}-agonists, or by NMDA (Andersson, 2001; Argiolas and Melis, 2005) The inhibitory effect of NOS inhibitors was not observed when these compounds were injected concomitantly with L-arginine, the substrate for NO. This was soon confirmed by other studies showing that NO donors, but also high doses of L-arginine, injected into the PVN induce penile erection episodes indistinguishable from those seen after the dopamine agonists NMDA and oxytocin (Argiolas, 1994; Melis and Argiolas, 1995; Melis et al., 1997a,b,d). The mechanism by which these compounds induce penile erection is apparently secondary to the release of NO, which in turn causes the activation of oxytocinergic neurons. Direct measurements of NO in the MPOA showed NO release associated with copulatory behavior. Local administration of a NOS inhibitor decreased NO release and copulatory behavior (Sato et al., 1998, 1999). NO production increased in the PVN during noncontact erection and copulation (Melis et al., 1998). The PVN is one of the brain areas containing the highest levels of NOS, and the enzyme is present in the cell bodies of oxytocinergic neurons. The proerectile effect of NO donors is prevented by the injection of oxytocin antagonists into the lateral ventricles. The mechanism by which endogenous or exogenous NO activates oxytocinergic neurons to release oxytocin in brain areas distant from the PVN to facilitate penile erection is still unknown. Because guanylyl cyclase (GC) inhibitors (e.g., methylene blue) injected into the PVN fail to prevent drug-induced penile erection, and 8-bromo-cGMP injected into the PVN fails to elicit erections, it has been proposed that the mechanism of NO action is not associated with the activation of GC (Melis and Argiolas, 1997). The additional finding that the NO scavenger hemoglobin does not prevent penile erection despite its ability to reduce NO levels in the PVN suggests that NO acts as an intracellular rather than an intercellular modulator of erectile responses involving the PVN (Melis and Argiolas, 1997).

In the spinal cord, the distribution of NOS-containing neurons suggests that NO plays a role in spinal cord neurotransmission, including preganglionic sympathetic and parasympathetic, somatosensory, visceral sensory, and possibly motor pathways (Valtschanoff et al., 1992; Dun et al., 1993; Saito et al., 1994; Burnett et al., 1995). At the spinal cord level, the functional role of NO for erection is not known.

N. Prolactin

Hyperprolactinemia can depress sexual behavior and reduce sexual potency in men (Drago, 1984; Krüger et al., 2005) and depress genital reflexes in rats (Rehman et al., 2000). Rehman et al. (2000) demonstrated a central neurological effect of hyperprolactinemia on erectile function. Hypogonadism did not seem to contribute to impaired penile reflexes as documented by the fact that replacement of testosterone did not recover the depressed centrally mediated penile reflexes.

Short- and long-term central prolactin treatment in male rats, however, may have stimulatory and inhibitory effects on sexual behavior, respectively (Cruz-Casallas et al., 1999) Correspondingly, striatal dopaminergic activity was shown to be increased and decreased by short-term and 5-day central prolactin treatment (Cruz-Casallas et al., 1999), supporting the view that the effects of prolactin are associated with changes in striatal dopaminergic activity. Prolactin has been shown to inhibit the dopaminergic incertohypothalamic pathway to the MPOA (Lookingland and Moore, 1984). In humans, it is still unclear whether the negative effects of hyperprolactinemia on erectile function are mediated centrally by way of reduction in sexual interest and sex drive (Carani et al., 1996) or through a direct effect of prolactin on CC smooth muscle contractility. In dogs, a direct effect on the CC was suggested (Ra et al., 1996). In any case, the effect seemed independent of circulating testosterone levels and gonadal axis function (Sato et al., 1997).

O. Sexual Hormones

Androgens, particularly testosterone, have been shown to have both central and peripheral effects that can influence penile erection (Traish et al., 2007; Buvat et al., 2010). They are necessary (although not sufficient) for sexual desire in men, are essential in the maintenance of libido, and have an important role in regulating erectile capacity (Mills et al., 1996; Gray et al., 2005; Gooren and Saad, 2006; Traish et al., 2007; Buvat et al., 2010). In men with normal gonadal function, however, there is no correlation between circulating testosterone levels and measures of sexual interest, activity, or erectile function (Krause and Müller, 2000). After castration in the male or other causes leading to a reduction in androgen levels, there is generally a decline in libido, and sometimes in erectile and ejaculatory functions. Testosterone administration restores sexual interest and associated sexual activity in hypogonadal or castrated adult men (Skakkebaek et al., 1981; O'Carroll et al., 1985; Traish et al., 2007; Buvat et al., 2010). The

testosterone dose-response relationships for sexual function and visuospatial cognition differ in older and young men; higher testosterone doses are needed in the elderly for normal sexual functioning (Gray et al., 2005).

IV. Peripheral Regulation

As mentioned, penile erection is initiated after central processing and integration of peripherally and/or centrally generated stimuli. The different structures of the penis receive sympathetic, parasympathetic, somatic, and sensory innervation (Dail, 1993; Hedlund et al., 1999), and the nerves contain different transmitters. The nerve populations have been categorized as adrenergic, cholinergic, and nonadrenergic, noncholinergic (NANC). All types of nerves may contain more than one type of transmitter. Thus, NANC nerves can contain not only neuropeptides but also transmitters and transmitter/modulator generating enzymes, such as NOS and heme oxygenases (HO). NANC transmitters/modulators can also be found in both adrenergic and cholinergic nerves, which should make it more meaningful to define nerve populations based on their transmitter content. Thus, it seems that one important population of nerves in the CC contains not only ACh, but also NOS, vasoactive intestinal peptide (VIP), and neuropeptide Y (Hedlund et al., 1999, 2000a,b).

The nerves and endothelium of sinusoids and vessels in the penis produce and release transmitters and modulators that interact in their control of the contractile state of the penile smooth muscles (Fig. 3). In addition, they may also have other important functions.

V. Transmitters and Mediators

A. Noradrenaline

NA, released from adrenergic nerves, stimulates ARs in the penile vessels and CC, producing a contraction that involves Ca^{2+} entry through both L-type and 2-aminoethoxydiphenyl borate-sensitive receptor-operated channels, as well as Ca^{2+} sensitization mechanisms mediated by protein kinase C (PKC), tyrosine kinases, and Rho kinase. It is generally accepted that this tonic activity keeps the penis in the flaccid state (Andersson and Wagner, 1995; Simonsen et al., 2002; El-Gamal et al., 2006; Villalba et al., 2007, 2008; Prieto, 2008). Becker et al. (2000) found that in humans, penile erection was accompanied by a significant reduction of NA in cavernosal blood, whereas adrenaline concentration was increased.

Both α_1- and α_2-ARs have been demonstrated in human CC tissue (Prieto, 2008), but available information supports the view of a functional predominance of postjunctional α_1-ARs for contraction, whereas NA via prejunctional α_2-ARs may down-regulate not only its own release but also that of NO (Prieto, 2008). The mRNAs of all subtypes of α_1-ARs with a high affinity for prazosin (α_{1A}, α_{1B}, and α_{1D}) have been demonstrated in human CC tissue. However, Goepel et al. (1999) showed that expression of α_{1A}, α_{1B}, and α_{2A} receptor proteins

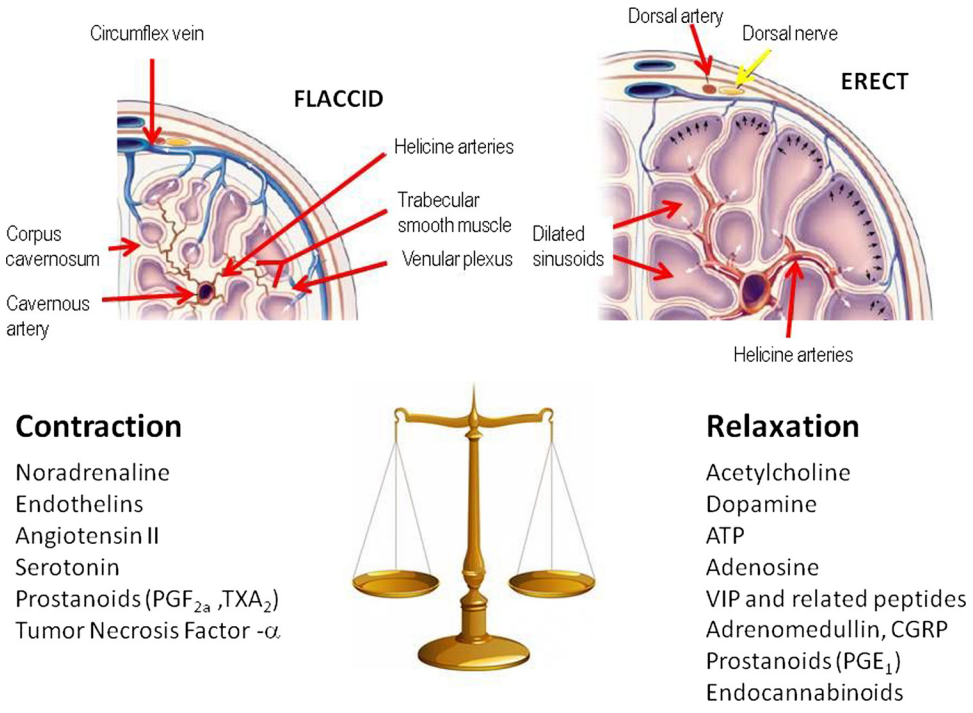

FIG. 3. In the penile vessels and the smooth muscle of the corpora cavernosa, the balance between contractant and relaxant factors controls the degree of tone of the penile vasculature and of the smooth muscle. This in turn determines the functional state of the penis: detumescence and flaccidity, tumescence and erection.

predominated and that the α_{1D}-AR is present only at the mRNA level. The functional α_1-AR proteins in human CC tissue were characterized by Traish et al. (1995a,b) using receptor-binding and isometric tension experiments. Their results demonstrated the presence of α_{1A}, α_{1B}, and α_{1D}-ARs, and they suggested that two or possibly three receptor subtypes mediate the NA-induced contraction in this tissue. An additional α_1-AR subtype with low affinity for prazosin, α_{1L}, which probably represents a conformational state of the α_{1A}-AR, has been suggested to be of importance in human penile erectile tissues. Morton et al. (2007) assessed the response of dorsal and cavernosal penile arteries on α-AR-selective agonists and antagonists in the rabbit. They found a predominant functional α_{1A}-AR population with little evidence of other α_1-AR subtypes in cavernosal arteries; there seemed to be evidence for the presence of α_2-ARs in the dorsal arteries providing nutritional supply. The authors concluded that α_1-AR antagonists with affinity for both α_{1A}-AR and α_2-AR would potentially have pro-erectile properties, with the combination of these perhaps being the most effective. In rats, α_{1B}- and α_{1L}-AR subtypes seemed functionally relevant for erectile function (Sironi et al., 2000). However, Hussain and Marshall (1997) found that the α_{1D}-AR predominated in several systemic rat vessels in vitro, and Mizusawa et al. (2002b) likewise found evidence for a functional predominance of the α_{1D}-AR subtype in rat erectile tissue. Sironi et al. (2000) suggested that antagonists with a subtype selectivity for α_{1B}- and/or α_{1L}-ARs could offer advantages in the treatment of ED. However, the distribution of α_1-AR subtypes in the penis and systemic vessels may not be the same in rabbit, rats, and humans (Rudner et al., 1999).

Expression of mRNA for α_{2A}-, α_{2B}-, and α_{2C}-ARs in whole human CC tissue has been demonstrated. Radioligand binding revealed specific α_2-AR binding sites, and functional experiments showed that the selective α_2-AR agonist 5-bromo-*N*-(4,5-dihydro-1*H*-imidazol-2-yl)-6-quinoxalinamine (UK14,304) induced concentration-dependent contractions of isolated strips of human CC smooth muscle (Gupta et al., 1998; Traish et al., 1997, 1998). Whether or not these α_2-ARs are of importance for the contractile regulation of tone in CC smooth muscle is still unclear. As mentioned, prejunctional α_2-ARs have been shown to inhibit stimulus-evoked release of NA from nerves in the human CC. Stimulation of prejunctional α_2-ARs in horse penile resistance arteries was also shown to inhibit relaxation-mediating NANC-transmitter release (Simonsen et al., 1997a,b; Prieto, 2008). This might a mechanism by which NA maintains detumescence.

B. Endothelins

Endothelins (ETs) have been demonstrated in penile erectile tissues and may have different roles in erectile function, including maintenance of CC smooth muscle tone (Andersson and Wagner, 1995; Andersson, 2001; Ritchie and Sullivan, 2011). In the endothelium of human CC tissue, intense ET-like immunoreactivity has been observed; immunoreactivity has also been observed in the CC smooth muscle. Binding sites for ET-1 have been demonstrated by autoradiography in the vessels and in CC tissue. Both ET_A and ET_B receptors have been found in human CC smooth muscle membranes, and it cannot be excluded that both receptor subtypes are functional (Andersson, 2001).

ET-1 potently (at least 2 to 3 -log units more potent than α_1-AR agonists) induces slowly developing, long-lasting contractions in different smooth muscles of the penis: CC, cavernosal artery, deep dorsal vein, and penile circumflex veins. Contractions can be evoked in human CC tissue also by ET-2 and ET-3, although these peptides are less potent than ET-1. The contractions induced by ET-1 seem to dependent on several mechanisms: transmembrane calcium flux (through voltage-dependent and/or receptor-operated calcium channels), mobilization of inositol 1,4,5-trisphosphate (IP_3)-sensitive intracellular calcium stores, and calcium sensitization through the Rho-Rho kinase pathway (Andersson and Wagner, 1995; Ritchie and Sullivan, 2011).

ETs may also function as modulators of the contractile effect of other agents (e.g., NA). Mumtaz et al. (2006) assessed the effect of ET-1 and its possible role in the α_1-AR pathway during the erectile process using organ bath studies on rabbit CC smooth muscle. ET_A receptors were found to play a greater role than ET_B receptors in the ET-1-induced contraction, but the α_1-AR-dependent pathway did not involve the ET_A or ET_B receptors. This does not exclude a positive interaction between the pathways (Andersson, 2003; Wingard et al., 2003). Wingard et al. (2003) showed in rat CC that ET-1 (in low concentrations) increased the effects of α_1-AR stimulation and caused a 4-fold increase in RhoA in the CC membrane fraction.

The role of ET_B receptors in the CC has not been clarified. ET_B receptor activation is known to possibly induce a NO-mediated decrease in penile vascular tone (Ari et al., 1996; Parkkisenniemi and Klinge, 1996). Filippi et al. (2003) studied the effects of hypoxia on the ET-1 sensitivity of CC and found that hypoxia caused an overexpression of ET_B receptors that was associated with a decreased contractile activity of ET-1 and an increased ET_B-mediated relaxation. Hypoxia also induced a time-dependent down-regulation of RhoA and Rho kinase expression. Filippi et al. (2003) concluded that these effects were counter-regulatory mechanisms that are switched on to decrease the contractile effect of ET-1 after more than physiological hypoxia, thus protecting the CC from protracted hypoxia.

Becker et al. (2001b) investigated the plasma ET-1-levels in 33 healthy adult men and in 25 patients with ED. In the healthy men, no changes in ET-1/ET-2 levels were observed in the systemic and cavernosal blood dur-

ing penile tumescence, rigidity, and detumescence. However, in the patients with ED, mean plasma ET-1/ET-2 levels during penile flaccidity and detumescence were found to be higher in the systemic circulation than in the cavernosal blood. However, Becker et al. (2001b) concluded that their data did not support speculations regarding the involvement of ET-1 in the pathophysiology of ED. El Melegy et al. (2005) found significantly greater mean plasma levels of ET-1 in the venous blood of patients with ED than in control subjects. They also found that patients with organic ED had significantly higher levels of ET-1 in both venous and cavernosal blood than those with psychogenic ED, and they suggested that ET-1 could be a clinical marker of diffuse endothelial disease manifested by ED.

Some evidence suggests that ETs play a pathophysiological role in various disease states (Ritchie and Sullivan, 2011). For example, in patients with diabetes mellitus and ED, increased plasma and CC levels of ET-1 have been demonstrated (Francavilla et al., 1997). Kendirci et al. (2007), studying the effects of long-term cocaine administration on erectile function in a rat model, found significantly increased plasma big-ET-1 levels in a cocaine treatment group compared with control animals. Cocaine administration significantly increased ET_A receptor expression in CC compared with saline controls, whereas ET_B receptor expression was not altered. Cocaine-treated rats also showed significantly decreased endothelial NOS (eNOS) expression and NO production. The authors concluded that cocaine administration significantly reduces erectile function in rats and that the pathophysiologic mechanisms probably involved increased plasma big-ET-1 levels, increased penile ET_A receptor expression, and reduced penile eNOS expression.

In addition to acting as long-term regulators of CC smooth muscle tone, ETs may modulate cellular proliferation and phenotypic expression (Andersson, 2001; Ritchie and Sullivan, 2011). ET-1 has been hypothesized to be directly involved in end-organ damage in salt-sensitive forms of hypertension. In support of this hypothesis, Carneiro et al. (2008b) found that activation of the ET-1/ET_A pathway contributed to mineralocorticoid hypertension-associated ED. ET_A receptor blockade may thus represent an alternative therapeutic approach for ED associated with salt-sensitive hypertension and in pathological conditions in which increased levels of ET-1 are present.

Even if much available in vitro information suggests that ETs may be of importance for erectile physiology and pathophysiology, the role of the peptides in erectile physiology/pathophysiology is unclear. So far, the only published pilot clinical study with selective ET_A receptor antagonists failed to show enhancement of erectile responses in men with mild-to-moderate ED (Kim et al., 2002). Thus, even if ETs may contribute significantly to the maintenance of the flaccid state, its primary role in human CC may not be as a contractile agent. The specific roles of the ET_A and ET_B receptors in the human CC need further clarification.

C. The Renin-Angiotensin System

There is evidence that a local renin-angiotensin system (RAS) exists within the CC (Becker et al., 2001c) and that several active peptides, particularly angiotensin II (Ang II), may be involved in the erectile mechanisms. Ang II signaling in the CC and its implications in ED have been reviewed in detail by Jin (2009). In human CC, production and secretion and of physiologically relevant amounts of Ang II was demonstrated by Kifor et al., (1997). Ang II was found mainly in endothelial cells lining blood vessels and smooth muscle bundles within the CC (Kifor et al., 1997). In vitro, Ang II contracted human (Becker et al., 2001c) and canine (Comiter et al., 1997) CC smooth muscle. In canine CC, the effect was increased by NOS inhibition (Comiter et al., 1997). Intracerebroventricular injection of Ang II caused contraction and terminated spontaneous erections in anesthetized dogs, whereas administration of losartan, selectively blocking Ang II receptors (type AT1), resulted in smooth muscle relaxation and erection (Kifor et al., 1997). In the rabbit CC, results were obtained suggesting involvement of the RAS system in the regulation of CC smooth muscle tone and that AT1 receptors were important for mediation of the response (Park et al., 1997, 2005). In humans, Becker et al. (2001a) showed that during detumescence, there is an increase in the level of angiotensin II in cavernosal blood compared with the levels in the flaccid state. In patients with organic ED, Ang II levels were higher than those in patients with psychogenic ED (El Melegy et al., 2005). There is also evidence that rats with experimental diabetes have increased levels of Ang II both in plasma and in CC (Chen et al., 2007).

Available evidence thus suggests that the main function of the RAS system is Ang II-mediated contraction, contributing to maintenance of the penis in a flaccid state. However, Ang II is not the only active peptide of the RAS (Kifor et al., 1997). The RAS system comprises two major arms: a vasoconstrictor/proliferative arm in which the main mediator is Ang II acting on AT1 receptors, and a vasodilator/antiproliferative arm in which the major effector is Ang-(1–7) acting via the G protein-coupled receptor Mas (Santos et al., 2003). The Ang-(1–7)-Mas axis may play an important role in penile erection. da Costa Gonçalves et al. (2007) documented the presence of Mas in rat CC and the effect of its stimulation by Ang-(1–7). They found that that Ang-(1–7) acts as a mediator of penile erection by activation of Mas and subsequent NO release. In the absence of Mas, erectile function was severely compromised, as demonstrated by a markedly depressed response to electrical stimulation of the major pelvic ganglion associated with penile fibrosis. Furthermore, the severely depressed erectile function of deoxycorticosterone acetate-salt hypertensive

rats was essentially normalized by Ang-(1–7) administration. They suggested that their data provided strong evidence for a previously unsuspected key role of Ang-(1–7) and its receptor Mas in erectile function.

It could be expected that drugs that reduce the formation or action of Ang II, such as angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor blockers (ARBs), should improve erectile responses. In spontaneously hypertensive rats, enalapril induced structural remodeling of the penile vasculature and ameliorated blood inflow to the CC (Hale et al., 2001). Captopril improved erectile function of spontaneously hypertensive-stroke prone and normotensive aged rats (Dorrance et al., 2002). A few clinical studies have suggested that treatment with ARBs or ACE inhibitors may improve erectile function and sexual performance in patients with hypertension and metabolic syndrome (Fogari et al., 2001; Baumhäkel et al., 2008). However, a large randomized placebo-controlled study on whether ED is predictive of cardiovascular events failed to reveal any significant effect of an ARB (telmisartan) or an ACE inhibitor (ramipril) on ED (Böhm et al., 2010).

Considering that both ACE inhibitors and ARBs increase Ang-(1–7) levels in plasma and tissue (Iusuf et al., 2008), da Costa Gonçalves et al. (2007) suggested that the beneficial effects of the RAS blockade on erectile function could be mainly mediated by Ang-(1–7). It is obvious that the role of the RAS system in the CC is more complicated that was previously believed. The RAS system may have a dual in erectile function: pro-detumescence mediated by the AngII-AT1 axis and proerection mediated by the Ang-(1–7)-Mas axis (da Costa Gonçalves et al., 2007).

D. Acetylcholine

The importance of parasympathetic nerves for producing penile erection is well established (Andersson and Wagner, 1995). Penile tissues from humans and several animal species are rich in cholinergic nerves (Hedlund et al., 1999, 2000b) from which ACh can be released experimentally by transmural electrical field stimulation. ACh released from these nerves acts on muscarinic receptors located on CC smooth muscle cells and on the endothelium of sinusoids and vessels. Four muscarinic receptor subtypes (M_1–M_4) were shown to be expressed in human CC (Traish et al., 1995c). The receptor on smooth muscle was suggested to be of the M_2 subtype (Toselli et al., 1994; Traish et al., 1995c), whereas that on the endothelium was of the M_3 subtype (Traish et al., 1995). ACh causes endothelium-dependent relaxation of CC, penile arteries, and circumflex and dorsal veins in vitro (Andersson, 2001). In isolated CC cells, carbachol consistently produces contraction. This means that relaxation induced by ACh can be produced either by inhibition of the release of contractant factors (e.g., NA) and/or by the release of relaxation-producing factors (e.g., NO) (Ayajiki et al., 2009).

It is important to stress that ACh also acts on nicotinic receptors (Bozkurt et al., 2007; Ozturk Fincan et al., 2010). The presence of neuronal nicotinic ACh receptors in rabbit CC tissue and possible mechanisms underlying the nicotine's potentiation of electrical field stimulation-induced relaxation were investigated by Bozkurt et al. (2007). They showed that nicotine acts on the nicotinic ACh receptors located on nitrergic nerves, thereby evoking release of NO from these nerve terminals. The ACh receptor subunits involved included $\alpha3$–$\beta4$, $\alpha4$–$\beta2$, and $\alpha7$ (Ozturk Fincan et al., 2010). Because most nitrergic nerves are cholinergic, it may be speculated that ACh, released by the parasympathetic stimulation causing erection, acts not only via stimulation of endothelial M_3 receptors releasing of NO, but also on prejunctional nicotinic receptors stimulating its own release. However, ACh may also produce penile tumescence and erection by inhibiting the release of NA through stimulation of muscarinic receptors on adrenergic nerve terminals. Because antimuscarinic drugs do not seem to affect erection, at least in humans (Andersson and Wagner, 1995), the NO-releasing effect of nicotinic receptor stimulation may be more important that previously recognized.

E. Dopamine

The importance of dopamine and dopamine receptors in the CNS for penile erection is well established. However, the role of dopamine receptors in the CC and penile vessels is less certain.

Hyun et al. (2002) found dopamine D_1 and D_2 receptor gene expression in rat CC. In situ hybridization signals for dopamine D_1 and D_2 receptor mRNAs were localized to CC and dorsal vessels, and Western blot analyses showed peripheral dopamine D_1 and D_2 receptor proteins. In immunohistochemical assays, peripheral dopamine D_1 and D_2 receptor proteins were detected in dorsal nerves, dorsal vessels, and CC smooth muscle of the rat penile tissues. d'Emmanuele di Villa Bianca (2005) also demonstrated that both D_1- and D_2-like receptors were expressed in the human CC. They concluded that apomorphine had a peripheral relaxant direct effect as well as an antiadrenergic activity and that human CC possessed more D_1-like (D_1 and D_5) than D_2-like (D_2, D_3, and D_4) receptors. Both D_1- and D_2-like receptors were mainly localized on smooth muscle cells, and the relaxant activity of apomorphine was most probably mediated by D_1-like receptors, partially through NO release from endothelium.

Apomorphine may thus not only amplify sexual and copulatory behavior but also, by a complementary role, amplify neurogenically mediated erections by acting in the periphery (El-Din et al., 2007). Matsumoto et al. (2005), investigating the role of peripheral dopamine receptors for regulation of penile erection, found that in the rat isolated CC, pre- and postjunctional effects of apomorphine seemed to involve not only dopamine D_1- and D_2-like receptors, but also α-ARs. However, they

also found that at relevant systemic doses of apomorphine, peripheral effects of the compound were unlikely to contribute to its proerectile effects in rats.

F. Serotonin

5-HT pathways in the brain are known to be involved in the induction of penile erections in rats (Andersson, 2001), and Kimura et al. (2008) presented evidence that the 5-HT_{2C} receptor in lumbosacral spinal sites mediates not only dopamine–oxytocin–5-HT action but also melanocortin action on penile erections. However, the importance of peripheral 5-HT receptors is less well established. Finberg and Vardi (1990) demonstrated an in vivo 5-HT-mediated inhibitory action on penile erection in rats as a result of vasoconstriction of the cavernosal arteries. In addition, Esen et al. (1997) showed that the in vitro 5-HT-mediated contractile response in human penile veins was augmented in patients with venoocclusive disease. The involvement of 5-HT_{1A} (Hayes and Adaikan, 2002; Furukawa et al., 2003), 5-HT_{1B} (Hayes and Adaikan, 2002) and 5-HT_{2A} receptors (Furukawa et al., 2003) in contracting CC smooth muscle was shown in animal studies. Furthermore, 5-HT_{1A}, 5-HT_{2A}, and 5-HT_4 receptors were implicated in human erection (Uckert et al., 2003; Lau et al., 2006). Lau et al. (2007) further confirmed that the peripheral 5-HT pathway may play a part in the erectile process via 5-HT_{2A} receptor-mediated contractile and 5-HT_3 receptor-mediated relaxant activities. Thus, it cannot be excluded that 5-HT, released from penile nerves, is a contractile neurotransmitter in the erectile process, the importance of which has to be established.

G. Vasoactive Intestinal Peptide and Related Peptides

The penis of humans as well as animals is richly supplied with nerves containing VIP and VIP-related peptides, such as pituitary adenylate cyclase-activating polypeptide (PACAP) (Dail, 1993; Hedlund et al., 1994, 1995). The majority of these nerves also contain immunoreactivity to NOS, and colocalization of NOS and VIP within nerves innervating the penises of both animals and humans has been demonstrated by many investigators (see Andersson, 2001). It seems that most of these NOS- and VIP-containing neurons are cholinergic, because they also contain vesicular ACh transporter (Hedlund et al., 1999), which is a specific marker for cholinergic neurons (Arvidsson et al., 1997). In patients with diabetes and ED, some investigators found a marked reduction of VIP-like immunoreactivity in nerves associated with the CC smooth muscle (Gu et al., 1984; Lincoln et al., 1987), but others did not (Haberman et al., 1991). In addition, the results of animal studies on the role of VIP in penises of diabetic animals are conflicting (Miller et al., 1995; Maher et al., 1996).

VIP receptors ($VPAC_1$ and $VPAC_2$) are considered to mediate the actions of the peptide (Fahrenkrug, 1993; Harmar et al., 1998). By binding and activating the $VPAC_2$ receptor, VIP participates in the erectile process via the activation of the adenylyl cyclase/cAMP pathway. VIP-related peptides (e.g., PACAP) also seem to act through the VIP receptors.

The stimulatory effect of VIP on adenylyl cyclase leads to an increase in cAMP, which in turn activates cAMP-dependent protein kinase. In corporal tissue from humans (Hedlund et al., 1995), rats, and rabbits (Miller et al., 1995), VIP increased cAMP concentrations without affecting the cGMP levels. Both VIP and PACAP have an inhibitory and relaxation-producing effect on strips of human CC tissue and cavernosal vessels in vitro, but it has been difficult to convincingly show that VIP released from nerves is responsible for relaxation of penile smooth muscle in vitro or in vivo (Andersson and Wagner, 1995). Hayashida et al. (1996) found no evidence for a role of VIP in the regulation of tone in the canine CC.

Kim et al. (1995) reported that in rabbit CC, a VIP antagonist inhibited electrically induced contractions, suggesting that the peptide was released from nerves during stimulation. VIP antiserum (Adaikan et al., 1986) and α-chymotrypsin (Pickard et al., 1993) reduced or abolished the relaxant effect of exogenous VIP on isolated human CC but had no effect on relaxation induced by electrical stimulation of nerves. VIP was shown to improve erectile function more significantly under hypogonadal than normal conditions (Zhang et al., 2011), mainly as a result of the higher expression of VPAC2, $G\alpha_s$, and lower expression of $G\alpha_i$ and phosphodiesterase (PDE) 3A in CC of castrated rats. The authors concluded that androgen may negatively regulate the erectile effect of VIP. However, in humans with prostate cancer, chemical castration did not influence VIP immunostaining of the CC (Cormio et al., 2005).

As mentioned, not only NOS but also other peptides seem to be colocalized with VIP, (e.g., peptide histidine methionine), which is derived from the same precursor as VIP, PACAP, and helospectin (Andersson, 2001). Even if Hedlund et al. (1995) demonstrated some of these peptides to be effective relaxants of human CC preparations, a role for them as neurotransmitters and/or neuromodulators has yet to be demonstrated. It has been suggested that PACAP may serve as a sensory transmitter (Fahrenkrug, 2001).

The cyclic peptide urotensin II was identified as the natural ligand of an orphan G-protein-coupled receptor (UT receptor). Urotensin II and UT receptors are expressed in a variety of peripheral organs and especially in cardiovascular (CV) tissue but also on the endothelium of human CC. Urotensin caused an caused an endothelium- and NO-dependent relaxation in vitro and in vivo (d'Emmanuele di Villa Bianca, 2010). It was suggested that urotensin II and the UT receptor could be involved in the endothelial NO pathway of human CC and in erectile function.

The extent to which VIP or any of the other peptides demonstrated in the penis have an important role as a

neurotransmitter or modulator of neurotransmission has not been established. Their physiological role in penile erection and in ED remains to be settled, and if and to what extent they can be useful targets for ED treatment is unclear. So far, VIP receptors in the penis have been demonstrated to be a promising therapeutic target (section VIII.C).

H. Prostanoids

Human CC tissue has the ability to synthesize various prostanoids and the additional ability to metabolize them locally (Khan et al., 1999; Minhas et al., 2000). The production of prostanoids can be modulated by oxygen tension and suppressed by hypoxia. Corresponding to the five primary active prostanoid metabolites (PGD_2, PGE_2, $PGF_{2\alpha}$, PGI_2, and thromboxane A_2), there are five major groups of receptors that mediate their effects—the DP, EP, FP, IP, and TP receptors, respectively. cDNAs encoding representatives of each of these groups of receptors have been cloned, including several subtypes of EP receptors. Penile tissues may contain most of these groups of receptors; however, their role in penile physiology is still far from established (Khan et al., 1999; Minhas et al., 2000). Prostanoids may be involved in contraction of erectile tissues via $PGF_{2\alpha}$ and thromboxane A_2, stimulating TP and FP receptors and initiating phosphoinositide turnover, as well as in relaxation via PGE_1 and PGE_2, stimulating EP receptors (EP2/EP4) and initiating an increase in the intracellular concentration of cAMP. Prostanoids may also be involved in the inhibition of platelet aggregation and white cell adhesion, and some evidence suggests that prostanoids and transforming growth factor-β1 may have a role in the modulation of collagen synthesis and in the regulation of fibrosis of the CC (Moreland et al., 1995). Brugger et al. (2008) characterized the pharmacological and physiological activity of novel subtype-selective EP and DP receptor agonists using isolated human and rabbit penile cavernosal tissue in organ baths and in vivo measurements of intracavernosal pressure in rats and rabbits. They found no consistent correlation between the pharmacological profile (receptor binding and second messenger assays) of the EP agonists and their effect on cavernosal tissue tone. However, they found that a potent DP1-selective agonist, AS702224 (Woodward et al., 2011), caused penile erection. They concluded that the DP1 receptor mediates relaxation in human cavernosal tissue and stimulates pro-erectile responses also in both rats and rabbits.

I. ATP and Adenosine

Based on the findings that ATP and other purines were shown to decrease both basal and phenylephrine-stimulated tension in isolated rabbit CC preparations, it was suggested that ATP is a NANC transmitter in the CC and that purinergic transmission may be an important component involved in the initiation and maintenance of penile erection (Tong et al., 1992; Wu et al., 1993). However, none of the purines tested facilitated or inhibited the response of CC smooth muscle to electrical field stimulation, and therefore their role may be in the modulation of erection rather than as neurotransmitters (Wu et al., 1993). ATP injected intracavernosally in dogs was found to produce increases in intracavernosal pressure and erection (Takahashi et al., 1992b). This effect, which was unaffected by atropine and hexamethonium, could be obtained without changes in systemic blood pressure. In addition, adenosine produced full erection on intracavernosal administration (Takahashi et al., 1992a).

The relaxant activity of ATP may be mediated either by its interaction with ATP receptors, or by adenosine generated through the endonucleotidase-mediated breakdown of ATP. Filippi et al. (1999) found that ATP acted as a potent and NO-independent relaxant agent of human and rabbit CC. They also showed that the ATP effect was partially attributable to the metabolic breakdown of ATP to adenosine but was also due to a direct stimulation of P2 receptors, seemingly different from the classic P2Yand P2X receptor subtypes. Shalev et al. (1999) showed that human CC strips can be relaxed by stimulation of P2Y purinoceptors via NO release. This relaxation was mediated by an endothelium-dependent mechanism. They suggested that purines may be implicated in physiological erection in man. Phatarpekar et al. (2010) concluded in a recent review that available evidence suggests possible roles of adenosine signaling in erection, ED, and priapism.

Adenosine produces its effect on target cells by binding to four specific G-protein-coupled receptors: A_1, A_{2A}, A_{2B}, and A_3. (Fredholm et al., 2011). Each receptor has a unique affinity for adenosine and a distinct cellular and tissue distribution. A_1 and A_3 receptors are coupled to adenylyl cyclase by the inhibitory G-protein subunit ($G\alpha_i$) and hence serve to lower intracellular levels of cAMP. A_{2A} and A_{2B} adenosine receptors are commonly coupled to adenylyl cyclase by the stimulatory G-protein subunit ($G\alpha_s$) and serve to increase intracellular cAMP (Dai et al., 2009).

As pointed out by Dai et al. (2009), adenosine has several features making it an excellent candidate for contributing to normal and abnormal penile erection: it is a potent vasodilator with a very short half-life ($<$10 s), and it generates erection via cyclic nucleotide second messengers. Adenosine-mediated cAMP induction activates protein kinase A and results in decreased calcium calmodulin-dependent myosin light-chain phosphorylation and enhanced smooth muscle relaxation (Lin et al., 2005). Studies in several animal species, including humans (Kiliç et al., 1994), showed that intracavernosal injection of adenosine resulted in tumescence and penile erection (Chiang et al., 1994; Noto et al., 2001). Theophylline, an adenosine receptor antagonist, inhibited adenosine-induced penile tumescence (Noto et al., 2001).

Adenosine was suggested to act through stimulation of receptors belonging to the A_{2A} subtype (Mantelli et al., 1995). More recently, Tostes et al. (2007) presented data suggesting that adenosine-induced relaxation in mouse CC is mediated through activation of both A_{2A} and A_{2B} adenosine receptors. Mice lacking adenosine deaminase (which is necessary for the breakdown of adenosine) showed priapic activity involving A_{2B} receptors (Mi et al., 2008). Wen et al. (2010) suggested that increased effects of adenosine via A_{2B} receptor signaling has an essential role in the pathogenesis of penile fibrosis. Consistent with these reports, it was demonstrated that ED in men in some cases may be due to endothelial A_{2B} receptor dysfunction (Faria et al., 2006). However, not all forms of ED are associated with impaired adenosine signaling. For example, Carneiro et al. (2008a) showed that adenosine actions are preserved in ED seen in obese and type II diabetic da/db mice, suggesting that increased CC responses to adrenergic nerve stimulation are not due to impaired negative modulation of sympathetic neurotransmission by adenosine in this diabetic model.

J. Nitric Oxide and cGMP Signaling

Synthesis and release of NO and the consequences of NO binding to soluble guanylyl cyclase are essential steps in the erectile process and have recently been reviewed in detail (Musicki et al., 2009). The constitutive forms of the enzyme, nNOS (NOS1) and eNOS (NOS3), are coupled to Ca^{2+} and calmodulin and are the principal NOS isoforms involved in the induction of penile erection, whereas inducible NOS (NOS2) is independent of Ca^{2+} and calmodulin and requires new protein synthesis (Arnal et al., 1999).

1. Nitric-Oxide Synthases in the Penis. An important role for NO in the relaxation of CC smooth muscle and vasculature is widely accepted (Andersson and Wagner, 1995; Andersson, 2001; Musicki and Burnett, 2006; Musicki et al., 2009). There seems to be no doubt about the presence of nNOS in the cavernosal nerves and their terminal endings within the CC, and in the branches of the dorsal penile nerves and nerve plexuses in the adventitia of the deep cavernosal arteries (Andersson, 2001). Both the nerves (nNOS) and the endothelium (eNOS) of the CC may be the source of NO. The relative contribution of the different forms of NOS to erection has not been definitely established.

A variant of nNOS (penile nNOS) has been identified as two distinct isoforms in the penis of rat and mouse, a full-length α splice form and a β splice form that lacks the N-terminal postsynaptic density 95/disc-large/zona occludens domain, important for protein-protein interactions. Evidence suggests that the α splice variant is active in NO formation at nerve terminals, whereas the functional role of the β variant in vivo is unclear and might not be substantial (Magee et al., 1996; Gonzalez-Cadavid et al., 1999, 2000). The findings of Hurt et al. (2006) confirmed that alternatively spliced forms of nNOS are major mediators of penile erection. Mice lacking both eNOS and nNOS have erections, show normal mating behavior, and respond with erection to electrical stimulation of the cavernosal nerves. We were surprised to find that isolated corporal tissue from both wild-type and NOS-deleted animals showed similar responses to electrical stimulation (Burnett et al., 1996; Hurt et al., 2006). Functional studies support the occurrence and importance of eNOS in human cavernosal tissue (Andersson and Wagner, 1995; Musicki and Burnett, 2006), and this seems to be the case also in rat (Cartledge et al., 2000b) and mouse (Mizusawa et al., 2001) CC.

Although the interplay between the NOS isoenzymes continues to be a matter of study, existing evidence points toward a model (Fig. 4) in which nNOS initiates the erectile response, which is then maintained and increased by eNOS activity (the latter being activated by shear stress) (Hurt et al., 2002, 2006; Musicki and Burnett, 2006; Bivalacqua et al., 2007b; Musicki et al., 2009). eNOS has an indispensable role in the erectile response, and its activity and endothelial NO bioavailability are regulated by multiple post-translational molecular mechanisms, such as eNOS phosphorylation, eNOS interaction with regulatory proteins and contractile pathways, and actions of reactive oxygen species (ROS). These mechanisms regulate eNOS-mediated responses under physiological circumstances and provide various mechanisms whereby endothelial NO availability may be altered in states of vasculogenic erectile dysfunction (ED).

The influence of androgens on erectile function might to an important extent be mediated by the NO/cGMP pathway (Andersson, 2001) even if non–NO-dependent pathways have been demonstrated (Reilly et al., 1997;

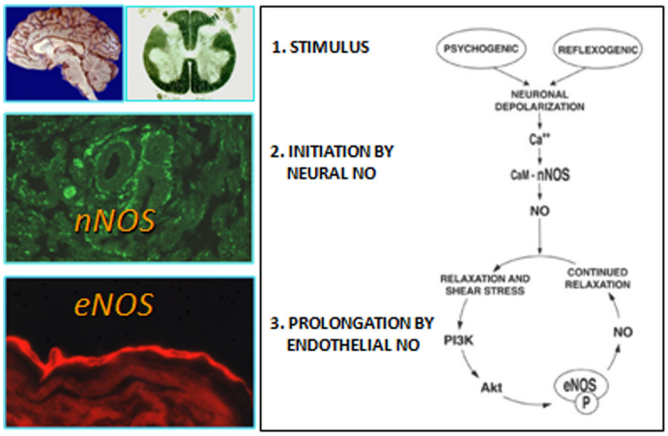

FIG. 4. Cooperation between nNOS and eNOS. Existing evidence points toward a model in which nNOS initiates the erectile response, which is then maintained and increased by eNOS activity (the latter being activated by shear stress). [Modified from Hurt KJ, Musicki B, Palese MA, Crone JK, Becker RE, Moriarity JL, Snyder SH, and Burnett AL (2002) Akt-dependent phosphorylation of endothelial nitric-oxide synthase mediates penile erection. *Proc Natl Acad Sci USA* **99:**4061–4066. Copyright © 2002 National Academy of Sciences, USA. Used with permission.].

Mills and Lewis, 1999; Mills et al., 1999). Castration of rats and treatment with the antiandrogen flutamide reduced constitutive penile NOS activity (Chamness et al., 1995; Lugg et al., 1996; Penson et al., 1996).

Compared with young rats, NOS-containing nerves, NOS mRNA expression, and NOS activity decreased in old animals (Garban et al., 1995; Carrier et al., 1997; Dahiya et al., 1997). ED associated with, for example, diabetes was found to be associated by a decreased nNOS content and activity in the rat CC (Vernet et al., 1995; Autieri et al., 1996; Rehman et al., 1997). In humans, the diabetic ED was suggested to be related to the effects of advanced glycation end products on NO formation (Seftel et al., 1997). Cartledge et al. (2000a) found in rats that glycosylated human hemoglobin impaired CC smooth muscle relaxation by generation of superoxide anions and extracellular activation of NO.

Angulo et al. (2006), evaluating the influence of PKC activity on penile smooth muscle tone in tissues from diabetic and nondiabetic men with ED, found that overactivity of PKC in diabetes is responsible for enhanced contraction and reduced eNOS-dependent relaxation of human CC smooth muscle.

2. Guanylyl Cyclases. The GCs, comprising both membrane-bound (particulate, pGC) and soluble isoforms (sGC), are expressed in nearly all cell types (Lucas et al., 2000). The GCs are stimulated by NO, natriuretic peptides, and other endogenous ligands (e.g., CO). CO, generated via heme oxygenase-mediated degradation of cellular heme, also stimulates sGC, albeit to a lesser extent than NO (Friebe et al., 1996).

Kim et al. (1998) demonstrated production of cGMP by pGC in the CC membranes of rabbit and rat stimulated by C-type natriuretic peptide 1–22 (CNP), atrial natriuretic peptide 1–28 (ANP), and brain natriuretic peptide 1–26 (BNP). In addition, CNP, but not ANP, relaxed precontracted isolated preparations of rabbit CC. Aizawa et al. (2008) investigated the effects of ANP, BNP, and CNP on intracavernosal pressure and systemic blood pressure in conscious, free-moving rats. They found that erectile responses could be initiated by ANP, by BNP, and less effectively by CNP. ANP and BNP have a high affinity for GC-A, suggesting that this receptor is involved in the responses.

Küthe et al. (2003) studied the expression of GC-B, a receptor of CNP in the human CC. mRNA transcripts were detected encoding for GC-B, and the expression was verified at the protein level by immunohistochemistry that demonstrated GC-B in CC and helical artery smooth muscle cells. CNP increased intracellular cGMP. In organ bath studies with CC muscle strips, CNP caused smooth muscle relaxation. It was concluded that CNP and its receptor might have a role in the induction of penile erection. A relaxant effect of ANP and uroguanylin was demonstrated in strips of human CC by Sousa et al. (2010). They found that uroguanylin relaxed the strips by a GC and K_{Ca}-channel-dependent mechanism and suggested that the natriuretic peptide receptors are potential targets for the development of new drugs for the treatment of ED. However, in the penis, sGC is probably the most important receptor for NO as a signaling molecule. The enzyme, which catalyzes the conversion of GTP into cGMP, consists of two different subunits and contains a prosthetic heme group that mediates up to 400-fold activation by NO. Nimmegeers et al. (2008) assessed the functional importance of the $sGC\alpha_1\beta_1$ isoform in CC from male $sGC\alpha_1(-/-)$ and wild-type mice. The relaxation to endogenous NO (from acetylcholine, bradykinin, and electrical field stimulation) was nearly abolished in the $sGC\alpha_1(-/-)$ CC. In the $sGC\alpha_1(-/-)$ mice, the relaxing influence of exogenous NO (from sodium nitroprusside and NO gas), 3-(4-amino-5-cyclopropylpyrimidine-2-yl)-1-(2-fluorobenzyl)-1*H*-pyrazolo[3,4-*b*]pyridine (BAY 41-2272; NO-independent sGC stimulator), and methyl-(2-(4-aminophenyl)-1,2-dihydro-1-oxo-7-(2-pyridinylmethoxy)-4-(3,4,5-trimethoxyphenyl))-3-isoquinoline carboxylic acid, sulfate salt (T-1032; phosphodiesterase type 5 inhibitor) were also significantly decreased. It was concluded that the $sGC\alpha_1\beta_1$ isoform is involved in CC smooth muscle relaxation in response to NO and NO-independent sGC stimulators.

3-(5′-Hydroxymethyl-2′-furyl)-1-benzylindazole (YC-1) was shown to elicit a direct activation of sGC (Fig. 5) by allosterically increasing the affinity for GTP and increasing the maximal enzyme activity, leading to increased cGMP levels in smooth muscle cells (Mülsch et al., 1997; Friebe and Koesling, 1998). Moreover, YC-1 caused a large activation in the presence of the NO donor sodium nitroprusside, which led to a remarkable 2200-fold stimulation of the human recombinant sGC (Lee et al., 2000). YC-1 caused concentration-dependent relaxant responses in NA-contracted rat CC preparations, and enhanced responses to electrical field stimulation. YC-1 also enhanced the relaxant response induced by carbachol. In vivo, YC-1 not only elicited dose-dependent erectile responses when administered intracavernosally but also increased the effects on intracavernosal

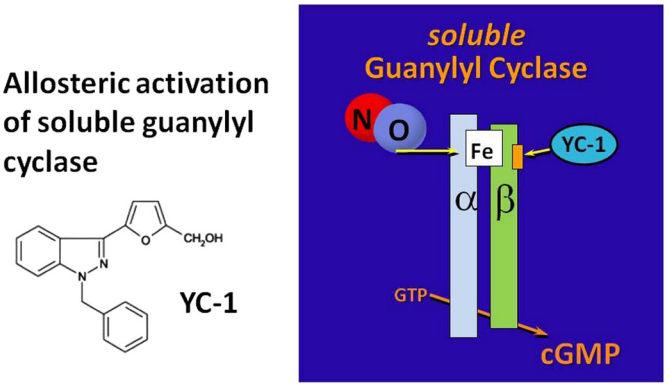

FIG. 5. Soluble guanylyl cyclase exists as a heterodimer of α (82 kDa) and β (70 kDa) subunits and has a catalytic site and two allosteric sites. One allosteric site is defined by the NO binding site (the heme; Fe), and the second is represented by the binding of YC-1. Agents such as YC-1 can activate sGC after binding to the allosteric site in the enzyme, leading to an increase in the intracellular concentration of cGMP, relaxation of cavernosal tissue, and facilitation of penile erection in vivo.

pressure produced by stimulation of the cavernosal nerve (Mizusawa et al., 2002a). YC-1 was able to significantly augment the pro-erectile effects of a suboptimal dose of apomorphine (Hsieh et al., 2003).

The pyrazolopyridine derivative BAY41-2272 was also found to stimulate sGC in a NO-independent manner and caused concentration-dependent relaxation of human and rabbit cavernosum (Kalsi et al., 2003) but induced only weak penile erections in conscious rabbits after intravenous and oral administration in the absence of an NO donor. However, the efficacy of BAY 41-2272 was potentiated by the simultaneous administration of SNP (Bischoff et al., 2003). In addition, other GC activators have been studied (Lasker et al., 2010). It has been pointed out that the efficacy of these sGC activators for ED has not been determined and that pilot studies are needed to assess their utility in the treatment of this disease. The use of both sGC activators and stimulators may be useful in conditions of altered heme conformation as well as in conditions in which NO synthesis is impaired (Lasker et al., 2010).

3. cGMP Signaling. The mechanisms involved in cGMP signaling have recently been extensively reviewed by Francis et al. (2010). Stimulation of GCs by NO and natriuretic peptides, and other endogenous ligands (e.g., CO), generates cGMP, which has influence on a number of vascular cell types and regulates vasomotor tone, endothelial permeability, cell growth, and differentiation, as well as platelet and blood cell interactions. There is evidence for reciprocal regulation of the NO-cGMP and natriuretic peptide-cGMP pathways and that one cGMP generating system may compensate for the dysfunction of the other (Kemp-Harper and Schmidt, 2009; Francis et al., 2010). cGMP signals via three main receptors in eukaryotic cells: ion channels, phosphodiesterases, and protein kinases (Lucas et al., 2000; Kemp-Harper and Schmidt, 2009; Francis et al., 2010). The molecular targets, which are activated by cGMP and finally produce relaxation of penile smooth muscle and erection, are still only partly known.

Three different cGMP-dependent protein kinases (cGKIα, cGK1β, and cGKII; also named PKGIα, PKGIβ, and PKGII) have been identified in mammals. Inactivation of cGKI in mice abolished both NO/cGMP-dependent relaxation of vascular and intestinal smooth muscle and inhibition of platelet aggregation, causing hypertension, intestinal dysmotility, and abnormal hemostasis (Pfeifer et al., 1998). CGKI-deficient [cGKI(−/−)] mice show a very low ability to reproduce. In CC tissue from these mice, the relaxant response to neuronally or endothelially released or exogenously administered NO was markedly reduced (Hedlund et al., 2000a). The expression of cGKI in penile tissue from cGKI(+/+) mice, as revealed by immunohistochemistry, was confined to the smooth muscle of the walls of the central and helicine arteries and to the smooth muscle of the trabecular septa surrounding the cavernosal spaces. This is in line with its presumed role in the erectile events. The total innervation (protein gene product 9.5 immunoreactivity) and distribution of nerve populations containing transmitters or transmitter-forming enzymes believed to be important in the regulation of tone in CC tissue (Andersson and Wagner, 1995) were similar in normal and cGKI-null mice (Hedlund et al., 2000).

Analysis of the NO/cGMP-induced relaxation clearly showed that cGKI is the major mediator of the cGMP signaling cascade in CC tissue. Its absence cannot be compensated for by the cAMP signaling cascade, which relaxes normal and cGKI-null penile erectile tissue to a similar extent. Taken together, these findings suggest that activation of cGKI is a key step in the signal cascade leading to penile erection.

The expression of cGKI was examined in CC specimens from patients with and without ED (Klotz et al., 2000). In all specimens of cavernosal tissue, a distinct immunoreactivity was observed in different parts and structures, with a high expression in smooth muscle cells of vessels and in the fibromuscular stroma. No clear immunoreactivity against cGKI was found in the endothelium. There was no distinct difference in immunoreactivity and cellular distribution between potent and impotent patients. This does not exclude that dysfunction of cGKI can be a cause of ED in humans, and that cGKI can be an interesting target for pharmacological intervention.

Bivalacqua et al. (2007a) investigated the expression of cGKIα (PKGIα) and cGKIβ (PKGIβ) in the CC and evaluated the effect of adenoviral gene transfer of cGKIα to the erectile compartment on EF in a rat model of diabetes. They found cGKIα and cGKIβ activities to be reduced in the erectile tissue of the diabetic rat. Supporting the role of cGK in the erectile process, gene transfer of cGKIα to the penis restored cGK activity and erectile function in vivo.

4. Phosphodiesterases. PDEs catalyze the hydrolysis of the second messengers cAMP and cGMP, which are involved in the signal pathways most important for relaxation of CC smooth muscle. The protein superfamily of cyclic nucleotide PDEs can be subdivided into at least 11 families of structurally and functionally related enzymes. More than 50 isoforms have been characterized so far, all differing in their primary structures, specificity for cAMP and cGMP, cofactor requirements, kinetic properties, mechanisms of regulation, and tissue distributions (Francis et al., 2010). Because of their central role in smooth muscle tone regulation and the considerable variation of PDE isoenzymes with respect to species and tissues, PDEs have become an attractive target for drug development. In human cavernosal tissue, at least 13 isoenzymes have been identified, including PDE3 (cGMP-inhibited cAMP PDE), PDE4 (cAMP-specific PDE), and PDE5 (cGMP-specific PDE) (Küthe et al., 1999, 2000, 2001). Functionally, PDE 3A and 5A seem to be the most important (Küthe et al., 1999, 2000, 2001;

Francis et al., 2010). Three of the PDE families (PDEs 5, 6, 9) have a >100-fold substrate preference for cGMP over cAMP as substrate and are therefore considered to be "cGMP-specific PDEs." PDE5 and PDE9 are the only "cGMP-specific PDEs" that are expressed in peripheral tissues; PDE6 is expressed in the retina (Francis et al., 2010).

PDE5, which is present in high concentrations in the smooth muscle of the CC, is the most therapeutically important because it is the target for the currently most widely used ED drugs, the PDE5 inhibitors. PDE5 is a homodimer containing two identical subunits with molecular mass of approximately 100,000 Da per subunit. Each of the two subunits has a catalytic domain and a regulatory domain. The catalytic domain, which is the target of PDE5 inhibitors, contains a single binding site for cGMP (Francis et al., 2010). Because they have structures similar to that of cGMP, sildenafil or other PDE5 inhibitors can also occupy the catalytic site, thus blocking access to cGMP competitively. However, sildenafil occupies the site approximately 1000 times more avidly than does cGMP, with the result that cGMP cannot bind to gain access to the catalytic machinery and accumulates in the smooth muscle cells of the CC. This leads to relaxation of the CC and penile erection (Francis et al., 2010). It is noteworthy that PDE5 seems to regulate sGC-derived but not pGC-derived cGMP, because ANP-mediated vasodilation is not enhanced in vitro or in vivo by the PDE5 inhibitor sildenafil (Kemp-Harper and Schmidt, 2009).

Lin et al. (2000, 2002, 2005) reported cloning of three PDE5 isoforms from human penile tissues. Two of the isoforms were identical to PDE5A1 and PDE5A2, respectively, which had previously been isolated from nonpenile tissues. The third isoform was novel and was called PDE5A3; this isoform was confined to tissues with a smooth muscle or cardiac muscle component.

The identification of the various PDE families has been paralleled by the synthesis of selective or partially selective inhibitors. Sildenafil, vardenafil, and tadalafil are highly selective inhibitors of PDE type 5, but several new compounds have been developed, some of which have significantly different structures (Boolell et al., 1996a,b; Francis and Corbin, 2005; Francis et al., 2010). They all enhance NO-mediated relaxation of CC from various species in vitro and in vivo by increasing the intracellular concentrations of cGMP by amplifying the endogenous NO-cGMP pathway (Kouvelas et al., 2009; Francis et al., 2010). The molecular mechanisms involved have been reviewed in detail elsewhere (Francis et al., 2010). PDE5 inhibitors are currently first-line treatment of ED (see section VIII.C), and several new selective PDE5 inhibitors are in different stages of development or have already been introduced clinically (Hatzimouratidis and Hatzichristou, 2008; Dorsey et al., 2010; Eardley et al., 2010).

Androgenic influences may affect PDE5 function in the penis (Morelli et al., 2004; Traish and Kim, 2005; Zhang et al., 2005), but the effect may be indirectly mediated by the influence of androgens on NOS function. These insights indicate that regulatory factors of PDE5 expression and activity in the penis critically determine the biological role of the enzyme.

5. Other Gaseous Mediators. Carbon monoxide (CO) and hydrogen sulfide (H_2S) are, together with NO, considered the principal peripheral proerectile gaseous transmitters that can be released mainly by cholinergic nerves and the sinusoidal endothelium to relax CC smooth muscle through the cGMP pathway. As mentioned, CO is generated via HO-mediated degradation of cellular heme and is able to stimulate sGC, but to a lesser extent than NO (Friebe et al., 1996).

A significant, positive effect of the HO/CO system on penile erection has been reported in several studies, and its potential role as molecular target in treatment of ED was reviewed by Shamloul (2009). None of the studies examined the role of the HO/CO system in aging animals, aging being considered the most important risk factor for ED. It was concluded that the HO/CO system might have a role in penile erection. However, further studies are needed to precisely delineate the importance of the HO/CO system in the physiology and pathophysiology of male function and sexual dysfunction.

L-Cysteine acts as a natural substrate for the synthesis of H_2S. Exogenous H_2S [administered as sodium hydrogen sulfide (NaHS)] or L-Cys caused a concentration-dependent relaxation of strips of human CC. In rats, the intracavernosal administration of either NaHS or L-Cys elicited penile erection (d'Emmanuele di Villa Bianca et al., 2010). These observations were suggested to indicate that a functional L-Cys/H_2S pathway may be involved in mediating penile erection in humans and other mammals.

K. Other Agents

1. Adrenomedullin, Calcitonin Gene-Related Peptide, Nociceptin. Adrenomedullin, a peptide belonging to the CGRP family, was first isolated from human phaeochromocytoma and has been suggested to serve as a circulating hormone regulating systemic arterial pressure (CGRP; Kitamura et al., 1993). The peptide has been demonstrated in the endothelial cells of human cavernosal tissue (Marinoni et al., 2005). Adrenomedullin inhibits the contraction of several types of smooth muscle cells not only through an increase in cAMP production, but also be stimulating the release of NO (Miura et al., 1995). Injected intracavernosally in cats, adrenomedullin caused increases in intracavernosal pressure and in penile length, an effect also found with CGRP (Champion et al., 1997a,b,c). Because the erectile responses to adrenomedullin or CGRP were unaffected by NOS inhibition with L-NAME or by K_{ATP} channel inhibition with glibenclamide, it was suggested that NO or K_{ATP} chan-

nels were not involved in the responses. The CGRP antagonist CGRP (8–37), at doses having no effect on the adrenomedullin response, reduced the responses to CGRP, suggesting that the peptides acted on different receptors. In the highest doses used, both adrenomedullin and CGRP reduced blood pressure (Champion et al., 1997a,b,c). Similar effects were found when adrenomedullin was injected intracavernosally in rats (Nishimatsu et al., 2001). In isolated precontracted rabbit CC strips, adrenomedullin caused a concentration-related relaxant effect (Gokce et al., 2004).

Bivalacqua et al. (2001a) used adenoviral gene transfer of prepro-CGRP to restore erectile function in the aged rat and found an improvement in erectile function. CGRP had been suggested previously to have potential for treating ED (Stief et al., 1990, 1991; Truss et al., 1994), but it does not seem likely that either adrenomedullin or CGRP will be clinically useful for treatment of ED.

Nociceptin is a 17-amino acid peptide that shares structural homology with the dynorphin family of peptides. It differs from other opioid peptides in that it does not have the NH_2-terminal residue, which is essential for activity at μ, δ, and κ opioid receptors (Henderson and McKnight, 1997; Calo' et al., 2000). The peptide is an endogenous ligand for the orphan opioid receptor that has been identified in several species: the human clone is called ORL1. It seems to be involved in a diversity of functions (Chiou et al., 2007) and has been shown to interfere with (inhibit) dopamine release in the brain (Olianas et al., 2008). Whether the latter action has any effects on erectile mechanisms or sexual behavior is not known.

Champion et al. (1997a) compared the erectile responses to intracavernosal nociceptin administration with those of a triple drug combination, VIP, adrenomedullin, and an NO-donor in cats. Nociceptin in doses of 0.3 to 3 nM elicited dose-related increases in intracavernosal pressure and penile length comparable with that of the triple drug combination, but the duration of the response was shorter. It does not seem likely that nociceptin has an important role in erectile mechanisms or that the ORL1 receptor is a reasonable target for drugs improving erectile function.

2. Endocannabinoids. There is little information concerning the peripheral effect of cannabinoids on CC tissue. Ghasemi et al. (2006) investigated the effect of the endogenous cannabinoid anandamide on the NANC relaxant responses to electrical field stimulation in isolated rat CC. They showed that anandamide has a potentiating effect on NANC-mediated relaxation through both CB1 and vanilloid receptors. Furthermore, they demonstrated that the NO-mediated component of the NANC relaxant responses to electrical stimulation is involved in this enhancement. The same group studied the effect of biliary cirrhosis on NANC-mediated relaxation of rat CC and the possible roles of endocannabinoid and NO systems in this model (Ghasemi et al., 2007). NANC-mediated relaxation was enhanced in CC strips from cirrhotic animals. Anandamide potentiated the relaxations in both groups. Either 1-(2,4-dichlorophenyl)-5-(4-iodophenyl)-4-methyl-*N*-(1-piperidyl)pyrazole-3-carboxamide (AM251; CB1 receptor antagonist) or capsazepine (transient receptor potential vanilloid 1 receptor antagonist), but not 6-iodo-2-methyl-1-[2-(4-morpholinyl)ethyl]-1*H*-indol-3-yl-(4-methoxyphenyl)methanone (AM630; CB2 receptor antagonist), prevented the enhanced relaxations of cirrhotic strips. Both the nonselective NOS inhibitor L-NAME and the selective neuronal NOS inhibitor N^{ω}-propyl-L-arginine inhibited relaxations in both groups, but the cirrhotic groups were more resistant to the inhibitory effects of these agents. Relaxations in response to sodium nitroprusside (NO donor) were similar in tissues from the two groups. The authors concluded that cirrhosis potentiates the neurogenic relaxation of rat CC, probably via the NO pathway and involving cannabinoid CB1 and transient receptor potential vanilloid 1 receptors.

Western blotting of CC tissues have demonstrated the existence of CB1 receptors in CC strips of rat and rhesus monkey (Gratzke et al., 2010b). In contrast to rat corporal tissue, relaxant responses to electrical stimulation were not altered in the presence of anandamide at 10 nM to 30 μM in monkey CC strips. Further studies are needed to elucidate the role of the endocannabinoid system in erectile tissue.

3. Tumor Necrosis Factor-α. Reactive oxygen species are important mediators of endothelial cell injury and dysfunction, which are the major triggers of pathophysiological processes leading to CV disease. A candidate factor in causing ROS production in endothelial cells is tumor necrosis factor α (TNF-α). TNF-α has been shown to play an important role in CV disease, mainly because of its direct effects on the vasculature (Chen et al., 2008; Zhang et al., 2009a), and may also be involved in ED, because high levels of TNF-α were demonstrated in patients with ED (Carneiro et al., 2010). TNF-α KO mice were found to exhibit changes in cavernosal reactivity that would facilitate erectile responses. Thus the animals showed decreased responses to adrenergic nerve stimulation and increased NANC- and endothelium-dependent relaxation that were associated with increased corporal eNOS and nNOS protein levels (Carneiro et al., 2009). TNF-α impaired endothelium-dependent and NO-mediated vasodilation in various vascular beds (Chen et al., 2008; Zhang et al., 2009a), and a key role for TNF-α in mediating endothelial dysfunction in ED has been suggested (Carneiro et al., 2009). Blockade of TNF-α actions (which is clinically possible) may theoretically represent an alternative therapeutic approach for erectile dysfunction, especially in pathological conditions associated with increased levels of this cytokine. Whether TNF-α can be a treatment alternative in such cases of ED remains to be established.

However, treatment with TNF-α antagonists may not be without problems. Experiences from treatment of disorders other than ED have shown that a third of patients do not respond to treatment for various reasons (Desroches et al., 2010).

VI. Impulse Transmission

A. Electrophysiology

In Vivo. CC tissue evokes electric waves that seem to be of diagnostic significance in evaluation of ED. However, it has been difficult to diagnose cavernosal nerve impairment in patients. Electrocavernosography has the potential to function as an investigative tool in diagnosing different types of ED (Wagner et al., 1989; Sasso et al., 1996; Shafik et al., 2004a,b). Advances in clinical electrophysiology now allow for consistent recording of intrapenile electrical activity. One promising method is evoked cavernosal activity, which can be recorded after a brief, startling stimulus (Yang et al., 2008; Yilmaz et al., 2009).

In Vitro. A variety of ion channels have been identified in CC smooth muscle cells, but there have been few electrophysiological investigations of whole corporal smooth muscle preparations (Andersson, 2001). In the proximal part of the rat corpus spongiosum (penile bulb), Hashitani (2000) demonstrated spontaneous action potentials in the inner muscle layer. On the other hand, no action potentials could be detected by electrophysiological investigation of cultured human CC smooth muscle cells (Christ et al., 1993). If this is valid also for the cells in vivo, it calls for an alternative mechanism for impulse propagation. Gap junctions may provide such a mechanism.

As underlined by Christ (2000), signal transduction in corporal smooth muscle is more a network event than the simple activation of a physiological cascade or pathway in individual myocytes. Gap junctions may contribute to the modulation of corporal smooth muscle tone and, thus, erectile capacity, and intercellular communication through gap junctions can provide the corpora with a significant "safety factor" or capacity for plasticity/adaptability of erectile responses.

Coordination of activity among the corporal smooth muscle cells is an important prerequisite to normal erectile function. The autonomic nervous system plays an important role in this process by supplying a heterogeneous neural input to the penis. For example, the activity of the various parts of the autonomic nervous system differs dramatically during erection, detumescence, and flaccidity (Becker et al., 2000). The role of the autonomic nervous system in normal penile function must be coordinated; the firing rate of the autonomic nervous system, myocyte excitability, signal transduction processes, and the extent of cell-to-cell communication between corporal smooth muscle cells must be carefully integrated to ensure normal erectile function.

VII. Excitation-Contraction Coupling

A. Ionic Distribution

The distribution of ions across the corporal smooth muscle cell membrane is important for the understanding of ion channel function. In conjunction with resting membrane potential of the corporal smooth muscle cell, this distribution ultimately determines the direction of ion flow during the opening of any given ion channel. These ionic gradients are maintained by a series of active membrane ion pumps and cotransporters, and are critical to the normal function of the corporal smooth muscle cell.

B. K^+ Channels

At least four distinct K^+ currents have been described in human corporal smooth muscle (Christ, 2000): 1) a calcium-sensitive maxi-K (i.e., BK_{Ca}) channel, 2) a metabolically regulated K channel (i.e., K_{ATP}), 3) a delayed rectifier K channel (i.e., K_{DR}), and 4) an "A" type K current. The BK_{Ca} channel and the K_{ATP} channel (Baukrowitz and Fakler, 2000; Archer, 2002) are the best characterized and probably the most physiologically relevant.

The distribution of K^+ across the corporal smooth muscle cell membrane ensures that the opening of potassium channels will lead to efflux of K^+ from the smooth muscle cell, down the electrochemical gradient. The movement of positive charge out of the cell results in hyperpolarization, and an inhibitory effect on transmembrane Ca^{2+}-flux through voltage-dependent Ca^{2+} channels (VDCCs).

1. The BK_{Ca} Channel. The BK_{Ca} channel has been well characterized in both human and rat corporal smooth muscle (Wang et al., 2000; Archer, 2002). BK_{Ca} channels are formed by two subunits: a pore-forming α-subunit and a modulatory transmembrane β-subunit. BK_{Ca} channel mRNA and protein have been detected in both freshly isolated human corporal tissues and cultured corporal smooth muscle cells (Christ et al., 1999). Consistent with such observations, the single-channel conductance ($\approx$180 pS), whole-cell outward currents, and voltage and calcium sensitivity of the K_{Ca} channel are remarkably similar when comparing data collected with patch clamp techniques on freshly isolated corporal smooth muscle myocytes versus similar experiments on short-term explant cultured corporal smooth muscle cells (Fan et al., 1995; Lee et al., 1999a,b).

The BK_{Ca} channel seems to be an important convergence point in modulating the degree of corporal smooth muscle contraction. The activity of this channel is increased after cellular activation of either the cAMP pathway by 8-bromo-cAMP or PGE_1 (Lee et al., 1999a) or the cGMP pathway by 8-bromo-cGMP (Wang et al., 2000). It seems clear that the two most physiologically relevant endogenous second-messenger pathways act to modulate corporal smooth muscle tone (i.e., elicit relax-

ation), at least in part, via activation of the BK_{Ca} channel subtype. The resulting hyperpolarization, in turn, is coupled to decreased transmembrane calcium flux through L-type VDCCs and, ultimately, smooth muscle relaxation.

The functional role of BK channels in the CC was investigated by Werner et al. (2005), using a knock-out mouse lacking the *Slo* gene (*Slo*(−/−)) responsible for the pore-forming subunit of the BK channel. CCSM strips from *Slo*(−/−) mice demonstrated a four-fold increase in phasic contractions, in the presence of phenylephrine. Nerve-evoked relaxations of precontracted strips were reduced by 50%, both in strips from *Slo*(−/−) mice and by blocking BK channels with iberiotoxin in the *Slo*+/+ strips. Consistent with the in vitro results, in vivo intracavernosal pressure exhibited pronounced oscillations in *Slo*(−/−) mice, but not in *Slo*+/+ mice. Furthermore, intracavernosal pressure increases to nerve stimulation, in vivo, were reduced by 22% in *Slo*(−/−)mice. These results indicate that the BK channel has an important role in erectile function, and loss of the BK channel leads to erectile dysfunction. Supporting this view, intracavernosal injection of cDNA encoding the human BK channel led to a reversal of erectile dysfunction in aged or diabetic rats and in atherosclerotic monkeys (Christ et al., 1998; Christ et al., 2004, 2009). These studies support the idea that elevating BK_{Ca}-channel expression can restore erectile function after age- or disease-induced decline. Opening of BK_{Ca} channels would be an alternative way of restoring an insufficient erectile function (e.g., Boy et al., 2004). Kun et al. (2009) found that NS11021 (1-(3,5-Bis trifluoromethylphenyl)-3-[4-bromo-2-(1*H*-tetrazol-5-yl)-phenyl]-thiourea), a novel opener of BK_{Ca} channels caused relaxation of both intracavernosal arteries and CC strips primarily through opening of BK_{Ca} channels. It was also effective in facilitating erectile responses in anesthetized rats. These results suggest a potential for use of BK_{Ca} openers in the treatment of ED. However, so far no successful clinical results have been published.

2. The K_{ATP} Channel. Western blots on isolated tissue strips, and immunocytochemistry of cultured corporal smooth muscle cells, using antibodies to the K_{ATP} channel, have documented the presence of the K_{ATP} channel protein (Christ et al., 1999). K_{ATP} channels are thought to be composed of two proteins: (1) an inward-rectifying K+ channel subunit (Kir; serving as the channel pore) and (2) a sulfonylurea receptor (SUR). In the human corporal smooth muscle the K_{ATP} channel are composed of the heteromultimers of Kir6.1 and Kir6.2 (Insuk et al., 2003). Experiments on freshly isolated corporal smooth muscle cells have documented the presence of two distinct ATP-sensitive K^+ currents in cultured and freshly dissociated human corporal smooth muscle cells (Lee et al., 1999a). Consistent with observations at the single channel level, whole cell patch clamp studies documented a significant, glibenclamide-sensitive, increase in the whole cell outward K^+ currents in the presence of the K channel modulator levcromakalim (see Lee et al., 1999a). These data, ranging from the molecular, through the cellular and whole tissue levels, clearly document the presence and physiological relevance of the K_{ATP} channel subtype(s) to the modulation of human corporal smooth muscle tone. Several studies have documented that K channel modulators, putative activators of the K_{ATP} channel, elicit a concentration-dependent relaxation of isolated human corporal smooth muscle (Andersson, 1992, 2001). Although activation of the K_{ATP} channel has been suggested to be involved in the action of yohimbine (Freitas et al., 2009), phentolamine (Silva et al., 2005) and testosterone (Yildiz et al., 2009) on cavernosal tissue, the importance of this contribution to the total effects of these agents on erectile mechanisms has not been established.

3. Calcium Channels. The distribution of Ca^{2+} ions across the CC smooth muscle cell membrane ensures that opening of Ca^{2+} channels will lead to influx of Ca^{2+} ions into the CC smooth muscle cell down their electrochemical gradient. The movement of positive charge into the smooth muscle cell has the opposite effect of the movement of K^+ out of the cell, and therefore, will lead to depolarization. Several studies have documented the importance of continuous transmembrane Ca^{2+} influx through L-type voltageVDCCs to the sustained contraction of human CC smooth muscle (Andersson, 2001). There seems to be only one published report of inward Ca^{2+} currents in CC smooth muscle using direct patch clamp methods (Noack and Noack, 1997). However, much of the most compelling mechanistic data concerning the role of Ca^{2+} channels in modulating human CC smooth muscle tone have been established using digital imaging microscopy of Fura-2 loaded cultured CC smooth muscle cells. These studies have provided strong evidence for the presence and physiological relevance of transmembrane Ca^{2+} flux through the L-type voltage-dependent calcium channel in response to cellular activation with e.g., ET-1 ($ET_{A/B}$ receptor subtype) and phenylephrine (α_1-adrenergic receptor subtype)(Christ et al., 1992b; Zhao and Christ, 1995; Staerman et al., 1997). Nifedipine-sensitive Ca^{2+} currents have been recorded from isolated rabbit CC smooth muscle cells (Craven et al., 2004), suggesting that the individual cells may be capable of generating action potentials by the opening of L-type Ca^{2+} channels.

The fact that inhibitors of L-type voltage-dependent calcium channels inhibit spontaneous contractions in isolated strips of CC is consistent with the idea that Ca^{2+} influx via this pathway is important for tone generation. However, the ability of human, rabbit and rat CC to develop phasic contractions and phasic electrical activity (e.g., Hashitani et al., 2005), suggests that the intracellular Ca^{2+} levels are oscillatory. This was confirmed in a study by Sergeant et al. (2009) in which spontaneous Ca^{2+} waves were shown to be generated

both in individual smooth CC muscle cells and across intact slices of CC tissue, where bursts of Ca^{2+} signals could be seen to trigger both phasic and tonic components of contraction. This "pacemaker" activity is likely to be of primary importance to the normal function of the CC, as it was shown to be associated with tissue contraction and inhibited by the NO/cGMP pathway.

McCloskey et al. (2009) studied VDCCs in rabbit CC myocytes dispersed enzymatically for patch clamp recording and confocal Ca^{2+} imaging. They found that the isolated myocytes developed robust VDCCs that could be separated into two components, one an L-type Ca^{2+}current, and the other a putative T-type current. The L-current facilitated conversion of local Ca^{2+} events into global Ca^{2+} waves, whereas the putative T-current seemed to play little part in this process. These findings confirm those of Zeng et al. (2005) demonstrating that human CC cells express T-type (α2G) Ca^{2+} channels that are involved in maintaining [Ca^{2+}] homeostasis.

4. Chloride Channels. The contribution of chloride channels/currents to the modulation of human corporal smooth muscle tone is less well understood than that of the other ion channels. Studies of calcium-activated Cl^- (Cl_{Ca}) function in the smooth muscle of CC have shown these channels to be involved in both the maintenance of spontaneous tone and the contractile response to noradrenaline and other agonists (Fan et al., 1999; Karkanis et al., 2003; Craven et al., 2004; Williams and Sims., 2007; Chu and Adaikan, 2008; Chung et al., 2009b). Karkanis et al. (2003) demonstrated an excitatory calcium Cl_{Ca} current in both human and rat corporal myocytes. This current was activated by agonist-induced Ca^{2+} release from stores and also occurs as spontaneous transient currents, which are typically caused by Ca^{2+} sparks. Cl_{Ca} channel blockers enhanced and prolonged the rise in pressure after cavernosal nerve stimulation, indicating that Cl^- current contributes to the regulation of intracavernosal pressure. Craven et al. (2004) proposed that Ca^{2+} activated Cl^- currents underlie detumescent tone in the CC, and modulation of this mechanism by the NO-cGMP pathway is important during penile erection. In support of these observations, Williams and Sims (2007) demonstrated that that Ca^{2+} sparks in CC arise from Ca^{2+} release through ryanodine receptors and give rise to Cl_{Ca} current. They also showed physiological regulation of spark frequency with depolarization as a result of voltage-dependent Ca^{2+} entry. These results were confirmed by Sergeant et al. et al. (2009), revealing that each of the Ca^{2+} waves was associated with an inward current typical of the Ca^{2+}-activated Cl^- currents developed by these cells. The waves depended on an intact sarcoplasmic reticulum Ca^{2+} store, because they were blocked by cyclopiazonic acid and agents that interfere with ryanodine receptors and IP_3-mediated Ca^{2+} release. Chu and Adaikan (2008) underlined the importance of Cl^- currents as a mechanism in the maintenance of CC tone produced by adrenergic and various endogenous constrictors in strips of rabbit CC. They suggested that the modulation of Cl^{-1} current could be an attractive and effective approach to regulate penile erection. In rats, Chung et al. (2009b) performed an in vivo study of the functional role of chloride channels in regulating erectile activity and concluded that chloride channels may play an important role in the regulation of CC tone.

E. Contractile Machinery

1. Contraction. In general, smooth muscle contraction is controlled by Ca^{2+} and Rho kinase signaling pathways (Berridge, 2008). Changes in the sarcoplasmic Ca^{2+} concentration, and thereby in the contractile state of the smooth muscle cell, can occur with or without changes in the membrane potential (Somlyo and Somlyo, 1994, 2000; Stief et al., 1997; Berridge, 2008) (Fig. 6). Action potentials or long-lasting changes in the resting membrane depolarize the membrane potential, thus opening voltage-gated L-type Ca^{2+} channels (Kuriyama et al., 1998). Thus, Ca^{2+} enters the sarcoplasm driven by the concentration gradient and triggers contraction. Membrane channels other than Ca^{2+} channels may also induce changes in the membrane potential. Opening of K^+ channels can produce hyperpolarization of the cell membrane. This hyperpolarization inactivates the L-type calcium channels, resulting in a decreased Ca^{2+} influx and subsequent smooth muscle relaxation.

According to Berridge (2008), the rhythmical contractions of CC smooth muscle depend on an endogenous pacemaker driven by a cytosolic Ca^{2+} oscillator that is responsible for the periodic release of Ca^{2+} from the sarcoplasmic reticulum (intracellular compartment for Ca^{2+} storage). The periodic pulses of Ca^{2+} often cause membrane depolarization; this is not part of the primary activation mechanism, but has a secondary role to synchronize and amplify the oscillatory mechanism. Neurotransmitters and hormones act by modulating the frequency of the cytosolic oscillator.

The major mechanisms involved in smooth muscle contractions, not associated with changes in membrane potential, are the release of IP_3 and the regulation of Ca^{2+} sensitivity. Both mechanisms may be important for the activation of CC smooth muscle. With regard to the physiologically important phosphatidylinositol cascade, many agonists (e.g., α_1-AR agonists, ACh, angiotensins, vasopressin) bind to specific membrane-bound receptors that are coupled to phosphoinositide-specific phospholipase C via GTP-binding proteins. Phospholipase C then hydrolyzes phosphatidylinositol 4,5-biphosphate to 1,2-diacylglycerol (this activates PKC) and IP_3. The water-soluble IP_3 binds to its specific receptor on the membrane of the sarcoplasmic reticulum, thereby opening this Ca^{2+} channel. Because the Ca^{2+}concentration in the sarcoplasmic reticulum is approximately 1 mM, Ca^{2+} is thus driven into the sarcoplasm by the concentration gradient, triggering smooth-muscle contraction. This increase in

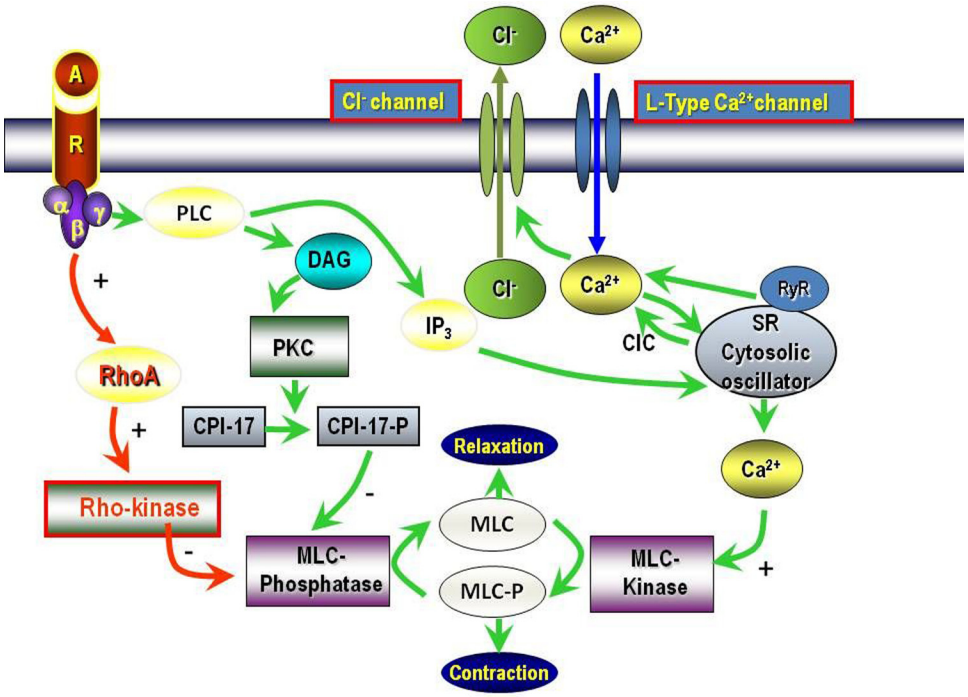

FIG. 6. Activation pathways in penile smooth muscle. According to Berridge (2008), for example, noradrenaline has two two main actions. It generates IP_3, which activates a cytosolic Ca^{2+} oscillator. It also activates the Rho/Rho kinase signaling pathway to increase the Ca^{2+} sensitivity of the contractile machinery. In addition, the Ca^{2+} transients activate Ca^{2+}-sensitive chloride channels that result in membrane depolarization to activate voltage-operated channels. This introduces Ca^{2+} to modulate the oscillator and also creates a flow of current to entrain the oscillatory activity of neighboring cells to account for the way these corpora cavernosa cells contract in near-unison with each other. A, agonist; R, receptor; PLC, phospholipase C; DAG, diacylglycerol; CPI-17, protein-kinase C-potentiated myosin phosphatase inhibitor; SR, sarcoplasmic reticulum; CIC, calcium-induced calcium release; RyR, ryanodine receptor.

sarcoplasmic Ca^{2+} concentration may activate a distinct Ca^{2+} release channel of the sarcoplasmic reticulum (i.e., the ryanodine receptor-operated channel), leading to a further increase in the Ca^{2+}concentration of the sarcoplasm (Somlyo and Somlyo, 1994, 2000; Karaki et al., 1997).

As in striated muscle, the amount of intracellular free Ca^{2+} is the key to regulation of smooth muscle tone. In the resting state, the level of sarcoplasmic free Ca^{2+} amounts to approximately ≈100 nM, whereas in the extracellular fluid, the level of Ca^{2+} is in the range of 1.5 to 2 mM. The cell-membrane Ca^{2+} pump and the Na^+/Ca^{2+} exchanger maintain this 10,000-fold gradient. Neuronal or hormonal stimulation can open Ca^{2+} channels, resulting in Ca^{2+} entry to the sarcoplasm down its concentration gradient. A rather modest increase in the level of free sarcoplasmic Ca^{2+} by a factor of 3 to 5 up to 550 to 700 nM then triggers myosin phosphorylation and subsequent smooth-muscle contraction.

In the smooth muscle cell, Ca^{2+} binds to calmodulin, which is in contrast to striated muscles, where intracellular Ca^{2+} binds to the thin-filament-associated protein troponin (Chacko and Longhurst, 1994; Karaki et al., 1997). The Ca^{2+}-calmodulin complex activates myosin light-chain kinase (MLCK) by association with the catalytic subunit of the enzyme. The active MLCK catalyzes the phosphorylation of the regulatory light-chain subunits of myosin (MLC_{20}). Phosphorylated MLC_{20} activates myosin ATPase, thus triggering cycling of the myosin heads (cross-bridges) along the actin filaments, resulting in contraction of the smooth muscle. A decrease in the intracellular level of Ca^{2+} induces a dissociation of the Ca^2-calmodulin MLCK complex, resulting in dephosphorylation of the MLC_{20} by myosin light-chain phosphatase and in relaxation of the smooth muscle (Somlyo and Somlyo, 1994, 2000; Karaki et al., 1997; Hirano, 2007).

In CC smooth muscle, which unlike most smooth muscles spends the majority of its time in the contracted state, an overall myosin isoform composition was found that was intermediate between aorta and bladder smooth muscles, which generally express tonic- and phasic-like characteristics, respectively (DiSanto et al., 1998; Berridge, 2008). Smooth-muscle myosin is composed of a pair of myosin heavy chains and two pairs of myosin light chains (MLC17 and MLC20) that are intimately intertwined. It has been shown that myosin heavy chain pre-mRNA is alternatively spliced to generate isoforms known as SM-A and SM-B. The SM-B isoform is predominantly found in SMs that demonstrate a more phasic contractile nature (e.g., urinary bladder), whereas the SM-A isoform is found in more tonic-type SM (e.g., aorta). DiSanto et al. (1998) have demonstrated that the CC smooth muscle possesses a myosin isoform composition somewhat intermediate between bladder and aortic SM. Zhang et al. (2009b) investigated the effects of blebbistatin, a small cell-perme-

able molecule originally reported as a selective inhibitor of myosin II isoforms. Blebbistatin completely relaxed human CC precontracted with phenylephrine in a dose-dependent manner. Maximal ICP and ICP/mean arterial pressure were dose-dependently increased by intracavernosal blebbistatin injections. These results of Zhang et al., (2009b) suggested an important role for the smooth contractile apparatus in the molecular mechanism of erection and suggested the possibility of blebbistatin binding at myosin II as a therapeutic treatment for ED by targeting SM contractile pathways.

In smooth muscle, the force/Ca^{2+} ratio is variable and depends partly on specific activation mechanisms. For example, α-AR agonists induce a higher force/Ca^{2+} ratio than does a depolarization-induced increase (i.e., KCl) in intracellular Ca^{2+}, suggesting a "Ca^{2+}-sensitizing" effect of agonists. Furthermore, it has been shown that at a constant sarcoplasmic Ca^{2+} level, decrease of force ("Ca^{2+}-desensitization") can be observed. It has generally been accepted that the key player in "calcium sensitization" is the MLC_{20} phosphorylation-dependent mechanism. Thus the balance between pathways leading to an increase in MLC_{20} phosphorylation and those leading to a decrease in MLC_{20} phosphorylation determine the extent of the "calcium sensitization" (Hirano, 2007).

a. The RhoA/Rho kinase pathway. Calcium sensitization is brought about by agonist activation of heterotrimeric G protein-coupled receptors, leading to the exchange of GDP for GTP on the small monomeric GTPase RhoA. This event elicits activation of RhoA and is catalyzed by the guanine nucleotide exchange factors, which cause dissociation of RhoA from its binding partner, Rho-guanine dissociation inhibitor. As a result, RhoA translocates from the cytosol to the membrane, enabling the downstream activation of various effectors such as Rho kinase. Phosphorylation of the regulatory subunit of MLC phosphatase by Rho kinase causes inhibition of phosphatase activity, which enhances the contractile response at a constant intracellular calcium concentration (Hirano, 2007).

This calcium-sensitizing RhoA/Rho kinase pathway may play a synergistic role in cavernosal vasoconstriction to maintain penile flaccidity (Andersson, 2003; Jin and Burnett, 2006). Although Rho kinase protein and mRNA have been detected in CC tissue, the exact role of Rho kinase in the regulation of CC tone has not been established. Using the Rho kinase antagonist *trans*-4-[(1*R*)-1-aminoethyl]-*N*-4-pyridinylcyclohexanecarboxamide dihydrochloride (Y-27,632) Chitaley et al. (2001) found that Rho kinase antagonism stimulated rat penile erection independently of NO and suggested that this principle could be a potential alternate avenue for the treatment of ED. Increased RhoA/Rho kinase activity may lead to abnormal contractility of the CC and contribute to the pathogenesis of diseases such as diabetes and hypertension, and possibly to other conditions associated with ED, such as hypogonadism and aging (Andersson, 2003; Jin et al., 2006). Several studies have suggested that NO inhibits RhoA/Rho kinase activity (Sauzeau et al., 2000, 2003; Sawada et al., 2001). Bivalacqua et al. (2007a) evaluated the regulatory influence of endothelial NO on the basal functional states of the NO and RhoA/Rho kinase signaling pathways in the penis using eNOS mutant mice and eNOS gene transfer technology. They found that eNOS in the CC of mutants had significant reductions in NOS activity, cGMP concentration, cGK activity, Rho kinase activity, and *p*-myosin phosphatase target-1 expression with no significant changes in activated RhoA or in RhoA and Rho kinase-α and -β protein expressions. After eNOS gene transfer to mutant animals, Rho kinase-β and p-myosin phosphatase target-1 expressions and total Rho kinase activity were significantly increased from baseline levels. They concluded that endothelial NO has a role in the penis as a regulator of the basal signaling functions of the NO and RhoA/Rho kinase erection mediatory pathways. Priviero et al. (2010), hypothesizing that basal release of NO from endothelial cells modulates contractile activity in the CC via inhibition of the RhoA/Rho kinase signaling pathway, arrived at a similar conclusion. Based on experiments on eNOS and nNOS KO mice, they suggested that there is a basal release of NO from endothelial cells, which inhibits contractions mediated by the RhoA/Rho kinase pathway and modulates the expression of proteins related to this pathway in mouse CC.

Several studies have shown that in pathological conditions, there is an imbalance in favor of increasing the RhoA/Rho kinase pathway [e.g., diabetic rats (Bivalacqua et al., 2004) and aging (Jin et al., 2006)]. One of the proposed mechanisms responsible for diabetes-related ED is overactivity of RhoA/Rho kinase signaling, as seen in experimental models of diabetes. Vignozzi et al. (2007) found that overexpression of RhoA/ Rho kinase signaling contributes to diabetes-related ED. They studied the effect of testosterone on RhoA/ Rho kinase signaling as responsiveness to the selective Rho kinase inhibitor Y-27,632 in vitro (rabbits) and in vivo (rats) with chemically induced diabetes. In both models, hypogonadism with reduced testosterone plasma levels was observed. Rho kinase 1 protein expression, as evaluated by Western blot analysis and immunohistochemistry analysis, was increased in the penes of diabetic animals and normalized by testosterone. It was concluded that treating hypogonadism in course of diabetes by normalizing RhoA/Rho kinase pathway up-regulation may maintain erectile function. Morelli et al. (2009) investigated whether atorvastatin, a statin, ameliorated diabetes-related ED. Streptozotocin- induced (8 weeks) diabetic rats and alloxan-induced (8 weeks) diabetic rabbits received atorvastatin (5 mg/kg daily) for the last 2 weeks. In both diabetic models, atorvastatin affected neither glycemia, lipid plasma levels, nor the hypogonadal state. In diabetic rats, atorvastatin ameliorated the erectile response to electrical stimulation of the cav-

ernosal nerve and normalized sildenafil effect on erectile function, which was markedly reduced by diabetes. In penile tissue from diabetic animals, atorvastatin completely restored the diabetes-induced hypersensitivity to Y-27632 and prevented RhoA membrane translocation/activation. Morelli et al. (2009) concluded that atorvastatin improved diabetes-related ED and restored sildenafil responsiveness, most probably by inhibiting RhoA/Rho kinase signaling. Gao et al. (2007) suggested that impaired erectile function with ageing in SD rats be associated with the imbalance between nNOS and Rho kinase activity and that the Rho kinase inhibitor, Y-27632, could improve the erectile function in old SD rats through adjusting this imbalance. Park et al. (2006) investigated whether long-term treatment with the oral Rho kinase inhibitor fasudil could prevent the development of both vasculogenic ED and pelvic atherosclerosis in a rat model. They found that the Rho/Rho kinase pathway is substantially involved in the development of ED and pelvic atherosclerosis, both of which could be prevented by long-termtreatment with fasudil.

In theory, suppression of an increased RhoA/Rho kinase activity is an attractive therapeutic principle in ED. However, the ubiquitous occurrence of the RhoA/Rho kinase pathway limits the use of Rho kinase inhibitors. If regulators of RhoA/Rho kinase uniquely expressed in penile tissue can be demonstrated, they may be targets for drugs. This will potentially lead to the development of new therapeutic agents for the treatment of ED.

2. Relaxation. As in other smooth muscles, CC smooth muscle relaxation is mediated via the intracellular cyclic nucleotide/protein kinase messenger systems. Via specific receptors, agonists activate membrane-bound adenylyl cyclase, which generates cAMP. cAMP then activates protein kinase A (cAK) and, to a lesser extent, cGK. ANF acts via pGC (Lucas et al., 2000), whereas NO stimulates sGC; both generate cGMP, which activates cGKI and, to a lesser extent, cAK. Activated cGKI and cAK phosphorylate phospholamban, a protein that normally inhibits the Ca^{2+} pump within the membrane of the sarcoplasmic reticulum. The Ca^{2+} pump is then activated and, consequently, the level of free cytoplasmic Ca^{2+} is reduced, resulting in smooth-muscle relaxation. Likewise, the protein kinases activate the cell-membrane Ca^{2+} pump, leading to a decreased sarcoplasmic Ca^{2+} concentration and to subsequent relaxation (Somlyo and Somlyo, 1994, 2000; Karaki et al., 1997; Berridge, 2008). Hashitani et al. (2002) suggested that decreasing the sensitivity of contractile proteins to Ca^{2+} may be the key mechanism of NO-induced relaxation in CC smooth muscle. In CC smooth muscle from the guinea pig, they found that in NA precontracted preparations, the NO donor SIN-1 inhibited 80% of the contraction and decreased $[Ca^{2+}]_i$ by 20%. In contrast, the calcium antagonist nifedipine, reduced $[Ca^{2+}]_i$ by 80%, whereas the level of contraction was decreased by only 20%. In high-potassium precontracted preparations, SIN-1 inhibited 80% of the contraction and reduced $[Ca^{2+}]_i$ by 20%.

VIII. Pharmacology of Current and Future Therapies

A. Risk Factors and Conditions Associated with Erectile Dysfunction

ED is often classified into four different types: psychogenic, vasculogenic or organic, neurologic, and endocrinologic (Lue, 2000; Lasker et al., 2010). It may be iatrogenic (e.g., after radical prostatectomy) or be a side effect of different pharmacological treatments (Erdemir et al., 2008; Kennedy and Rizvi, 2009). Although it is difficult to separate psychogenic factors from organic disease, vasculogenic ED was found to account for approximately 75% of patients with ED (National Institutes of Health Consensus Development Panel on Impotence, 1993). In general, patients with ED respond well (up to 70%; Hatzimouratidis and Hatzichristou, 2008) to the pharmacological treatments that are currently available. In those who do not respond, a structural alteration in the components of the erectile mechanism can be suspected. Various diseases commonly associated with ED can alter the mechanisms that control penile erection (see, e.g., Lewis et al., 2010; Albersen et al., 2011). Often, changes in the L-arginine/NO/cGMP system are involved. Other contributing factors could be the presence of endogenous NOS inhibitors (e.g., asymmetric dimethylarginine) and an up-regulation of arginase activity. High levels of asymmetric dimethylarginine have been demonstrated in multiple disorders in which NOS dysfunction has been implicated, including several of the disorders mentioned in the sections that follow, and increased arginase activity has been demonstrated in CC tissue in, for example, diabetes, aging, and smoking (Bivalacqua et al., 2001b; Imamura et al., 2007; Numao et al., 2007).

1. Endothelial Dysfunction. Endothelial dysfunction may be a main underlying factor for ED associated with many risk factors, such as hypertension, dyslipidemia, diabetes, depression, obesity, cigarette smoking, and the metabolic syndrome. Because a systemic endothelial dysfunction may functionally manifest itself early in the penile endothelium, the possibility arises that ED may be an early indicator of CV disease (Jackson et al., 2010; Shin et al., 2011).

2. Aging. Aging is an important risk factor for ED, and it has been estimated that 55% of men have ED at the age of 75 (Melman and Gingell, 1999; Johannes et al., 2000). Although age-related ED is attributed largely to increased oxidative stress and endothelial dysfunction in the penis, the molecular mechanisms underlying this effect are not fully defined. Aging in humans is also associated with several changes in arterial structure and function, part of them related to the decline of circulating levels of steroids (i.e., testosterone and estra-

diol) (Buvat et al., 2010). Such changes may be partly responsible for the lack of efficacy of ED treatments. There is evidence for involvement of the NO/cGMP system. Thus, Garban et al. (1995) found that the soluble NOS activity decreased significantly in penile tissue from senescent rats. Lower NOS mRNA expression was found in old rats than in young rats (Dahiya et al., 1997). In another rat model of aging, the number of NOS-containing nerve fibers in the penis decreased significantly, and the erectile response to both central and peripheral stimulation decreased (Carrier et al., 1997). In the aging rabbit, endothelium-dependent CC relaxation was attenuated; however, eNOS was up-regulated both in vascular endothelium and corporal smooth muscle (Haas et al., 1998). Johnson et al. (2011) evaluated whether eNOS uncoupling in the aged rat penis is a contributing mechanism. Their findings suggested that aging induces eNOS uncoupling in the penis, resulting in increased oxidative stress and ED.

3. Diabetes Mellitus. Diabetes mellitus is a significant risk factor for the development of ED (Saenz de Tejada and Goldstein, 1988; Melman and Gingell, 1999; Johannes et al., 2000; Saigal et al., 2006; Chitaley et al., 2009). According to the Massachusetts Male Aging Study, men with diabetes have a 28% prevalence of ED compared with 9.6% in the general population (Feldman et al., 1994). Men with diabetes have a 75% lifetime risk of developing ED and have earlier onset of ED compared with nondiabetics (Saigal et al., 2006). Many factors can contribute to diabetes-induced ED. Systemic effects of hyperglycemia and hypogonadism contribute to the development of impaired vasodilatory signaling, smooth muscle cell hypercontractility, and veno-occlusive disorder (Hidalgo-Tamola and Chitaley, 2009; Malavige and Levy, 2009). In isolated CC from patients with diabetes and ED, both neurogenic and endothelium-dependent relaxation was impaired (Saenz de Tejada et al., 1989); this was also found in rabbits in which diabetes was induced by alloxan (Azadzoi and Saenz de Tejada, 1992). Penile NOS activity and content were reduced in rat models of both type I and II diabetes with ED (Vernet et al., 1995). However, in rats with streptozotocin-induced diabetes, NOS binding increased (Sullivan et al., 1996), and NOS activity in penile tissue was significantly higher than in control rats, despite a significant degradation of mating behavior and indications of defective erectile potency (Elabbady et al., 1995). In humans, diabetic ED was suggested to be related to the effects of advanced glycation end products on NO formation (Seftel et al., 1997). The ability of diabetic tissue to convert L-arginine to L-citrulline via NOS was shown to be reduced, and it was suggested that an increased expression of arginase II in diabetic CC tissue may contribute to the ED associated with this disease (Bivalacqua et al., 2001b). Supporting this view, arginase II isoform deletion was shown to improve CC relaxation in mice with type I diabetes (Toque et al., 2011).

4. Atherosclerosis. Atherosclerosis is a significant risk factors involved in the development of vasculogenic ED. There is evidence of a strong link between ED and atherosclerosis (Maas et al., 2002; Grover et al., 2006; Jackson et al., 2006, 2010). ED and atherosclerosis share similar risk factors, and both conditions are characterized by endothelial dysfunction and impaired NO bioavailability. Recent data suggest that ED may serve as a sentinel marker that precedes the clinical diagnosis of atherosclerotic vascular disease (Montorsi et al., 2003; Gazzaruso et al., 2008). ED is an independent predictor of future adverse CV events; many men experience symptoms of ED years before their first diagnosis of CV disease. In a rabbit model of atherosclerotic ED (Azadzoi and Goldstein, 1992; Azadzoi et al., 1996), it was shown that chronic cavernosal ischemia impaired not only endothelium-dependent but also neurogenic CC relaxation and NOS activity (Azadzoi et al., 1998). There was also an increased output of constrictor eicosanoids in the CC. L-Arginine administration failed to improve CC relaxation, which was suggested to be due to impairment of the NOS activity and reduction of NO formation.

5. Hypercholesterolemia. Hypercholesterolemia was also found to impair endothelium-mediated relaxation of rabbit CC smooth muscle (Azadzoi and Saenz de Tejada, 1991; Azadzoi et al., 1998). Hypercholesterolemia did not affect NOS activity, but impaired endothelium-dependent but not neurogenic relaxation of rabbit CC tissue. Because the endothelium-dependent relaxation was improved after treatment with L-arginine, it was speculated that there was a deficient NO formation because of lack of availability of L-arginine in the hypercholesterolemic animals (Azadzoi and Saenz de Tejada, 1991; Azadzoi et al., 1998).

6. Smoking. Smoking is a major risk factor in the development of erectile dysfunction (Mannino et al., 1994; Gades et al., 2005; Shiri et al., 2005; Tostes et al., 2008). Clinical and basic science studies provide strong indirect evidence that smoking may affect penile erection by the impairment of endothelium-dependent smooth muscle relaxation or more specifically by affecting NO production via increased ROS generation. Whether nicotine or other products of cigarette smoke mediate all effects related to vascular damage is still unknown (Tostes et al., 2008).

7. Radical Prostatectomy. Despite advances in surgical technique, ED after radical prostatectomy, which remains the standard treatment for men with clinically localized prostate cancer, is a common complication. This is mainly attributed to temporary cavernosal nerve damage resulting in penile hypoxia, smooth muscle apoptosis, fibrosis, and veno-occlusive dysfunction (Magheli and Burnett, 2009).

B. Drugs for Treatment of Erectile Dysfunction

A wide variety of drugs has been recommended for treatment of ED, and the various options have been

extensively reviewed (Carson and Lue, 2005; Hatzimouratidis and Hatzichristou, 2008; Hellstrom, 2008; Dorsey et al., 2010; Eardley et al., 2010; Giovannoni et al., 2010; Hatzimouratidis et al., 2010; Albersen et al., 2011). Major advances have been made in our understanding of the mechanisms of penile erection and of drug action during the last decade. This may have led to a better and more detailed understanding of the rational basis for the therapeutic effects of the drugs, but few or no new drug principles have emerged and been introduced clinically (Andersson, 2001). Most clinical studies have confirmed the effects of the established drugs or drug principles in various patient populations with ED, documenting adverse effects and focusing on potential differences between drugs.

Currently used drugs can be classified in different ways. A pragmatic classification, used in most reviews, is by the route of administration.

C. Drugs for Nonintracavernosal Administration

1. Phosphodiesterase Inhibitors. Current ED treatment guidelines recommend PDE5 inhibitors as the first-line treatment (Hatzimouratidis et al., 2010). All the "major" PDE5 inhibitors, sildenafil, tadalafil, and vardenafil, have been assessed as effective and safe (Hatzimouratidis and Hatzichristou, 2008; Eardley et al., 2010). The overall efficacy rate of these drugs is approximately 60 to 70% but is considerably lower in some patient populations, such as those with severe neurological damage, ED after radical prostatectomy, diabetes, or severe vascular disease (Hatzimouratidis and Hatzichristou, 2008). The choice of a PDE5 inhibitor depends on several factors, including the frequency of intercourse and the patient's personal experience with the agent (Mirone et al., 2009).

PDE5 inhibitors were initially introduced as on-demand treatment; however, tadalafil has also been approved for continuous, everyday use in 2.5- and 5-mg doses. The actions of PDE5 inhibitors are often described in terms of selectivity (PDE5 versus other PDEs) and potency (the concentration needed for effect). PDE5 inhibitor selectivity is a key factor determining its adverse effect profile and may vary between agents (Table 1). Sildenafil and vardenafil cross-react slightly with PDE6. Because PDE6 predominates in the retina, this may explain the complaint of some patients that sildenafil or vardenafil may cause visual disturbances ($< 2\%$ of patients). Tadalafil to some extent cross-reacts with PDE11 (found in, for example, the heart, testes, and the anterior pituitary), but the consequences of this effect are unknown.

The common pharmacokinetic properties of PDE5 inhibitors [e.g., bioavailability, maximum plasma concentration (C_{max}), time (T_{max}) required for attaining C_{max}, and time required for elimination of half the inhibitor from plasma ($t_{1/2}$)] all influence efficacy (Table 2) (Gupta et al., 2005). Sildenafil, vardenafil, udenafil, and avanafil have broadly similar T_{max}, which predicts a similar time of onset of action. The $t_{1/2}$ values of tadalafil and udenafil are longer than those of the other PDE5 inhibitors, which could be caused by the slower intestinal absorption and/or slower degradation of these drugs by the liver, or by other factors. The extended $t_{1/2}$ of tadalafil provides a longer therapeutic effect, and this may also be the case for udenafil and SLx-2101. The C_{max} of vardenafil is significantly lower than that for sildenafil and tadalafil, probably depending on the lower bioavailability (Gupta et al., 2005). PDE5 inhibitors are degraded in the liver, and interactions with ketoconazole, for example (inhibiting CYP3A4) may prolong their effect duration. The duration of effect of a PDE5 inhibitor is not always reflected in its elimination from plasma. Molecular mechanisms that can contribute to this have been suggested (Francis et al., 2008). Thus, there may be a persistence of biochemical effects after the inhibitor is cleared from cells (memory effect). In addition, because inhibitors bind tightly to PDE5 in, e.g., muscle cells, this could significantly retard their exit from these cells and prolong their effects (Francis et al., 2008).

Common adverse events with PDE inhibitors include headache (10–16%), flushing (5–12%), dyspepsia (4–12%), nasal congestion (1–10%), and dizziness (2–3%) (Hatzimouratidis et al., 2010). Tadalafil may cause back pain/myalgia in 6% of patients. Adverse events are generally mild in nature and self-limited by continuous use, and the dropout rate due to adverse events is similar to that seen with placebo. Clinical trials and postmarketing data of all PDE5 inhibitors have demonstrated the drugs to be safe in patients with CV disease. Thus, no increase in myocardial infarction rates has been ob-

TABLE 1
Selectivity (potency) of phosphodiesterase type 5 (PDE5) inhibitors

Inhibitor	IC_{50} for PDE5	PDE Selectivity	Source
	nM		
Sildenafil	3.5–10	Low activity against PDE6	Francis et al. (2009)
Vardenafil	0.14–1	Low activity against PDE6	Francis et al. (2009)
Tadalafil	1.8–10	Low activity against PDE11	Francis et al. (2009)
Udenafil	8.2	Low activity against PDE3, PDE6	Doh et al. (2002)
Mirodenafil	0.33	"More selective than sildenafil"	Shin et al. (2006)
Avanafil	1	Low activity against PDE1, PDE6	Hellstrom (2008)
SLx-2101	0.24	N.A.	Sweetnam et al. (2006)

IC_{50}, half maximal inhibitory concentration; N.A., not available.

TABLE 2
Some pharmacokinetic characteristics of the "major" PDE inhibitors

	Sildenafil	Vardenafil	Tadalafil
Oral bioavailability, %	38–41	15 (8–25)	N.A.
T_{max}, h	1 (0.5–2)	0.7 (0.25–3)	2 (05–6)
C_{max}, μg/l (fasting)	560 (100 mg)	20.9 (20 mg)	378 (20 mg)
C_{max}, food effect	29% reduction	18% reduction	No effect
$t_{1/2}$, h	3–5	4–5	17.5
Cytochrome P450 isoenzyme	3A4, 2C9	3A4	3A4
Active metabolite	Yes	Yes	None

N.A., not available.
Modified from Gupta M, Kovar A, and Meibohm B (2005) The clinical pharmacokinetics of phosphodiesterase-5 inhibitors for erectile dysfunction. *J Clin Pharmacol* **45:**987–1003. Copyright © 2005 American College of Clinical Pharmacology, Inc. Used with permission.

served (Vlachopoulos et al., 2009). No PDE inhibitor has adversely affected total exercise time or time to ischemia during exercise testing in men with stable angina. In fact, they may improve exercise tests. PDE5 inhibitors may even be beneficial in CV disease (Takimoto et al., 2005), and sildenafil has been approved for treatment of pulmonary arterial hypertension (Galiè et al., 2005).

Nitrates are totally contraindicated with all PDE inhibitors due to unpredictable hypotension. The duration of interaction between organic nitrates and PDE inhibitors varies according to the PDE inhibitor and the nitrate. If a patient develops angina while using a PDE inhibitor, other antiangina agents may be used instead of nitroglycerin or until the appropriate time has passed (24 h for sildenafil or vardenafil and 48 h for tadalafil) (Vlachopoulos et al., 2009).

a. Sildenafil. The efficacy of sildenafil when taken on demand at doses of 25, 50, and 100 mg has been well documented in numerous reviews (Hatzimouratidis, 2006; Giuliano et al., 2010; Tsertsvadze et al., 2009; Eardley et al., 2010 and references therein). It is also clear that sildenafil is efficacious in men where the ED is a consequence of specific diseases such as diabetes, depression, spinal cord injury, multiple sclerosis, CV disease, and hypertension. It is effective in men with lower urinary tract symptoms and ED (Tsertsvadze et al., 2009; Eardley et al., 2010; Giuliano et al., 2010). Side effects do occur with sildenafil (most notably headache, flushing, indigestion, nasal congestion, and occasional visual changes), but providing that the drug is used in line with the labeling recommendations, there is no convincing evidence in the literature of any significant safety issue, including CV, visual, and aural safety (Tsertsvadze et al., 2009; Eardley et al., 2010; Giuliano et al., 2010).

b. Tadalafil. It is well documented that tadalafil is efficacious in the treatment of ED in the broad population when taken on demand at doses of 10 and 20 mg (Coward and Carson, 2008; Eardley et al., 2010; and references herein). There is also evidence confirming that tadalafil is efficacious when taken daily at doses of 2.5 and 5 mg. It was shown that daily use of 5- and 10-mg tadalafil for 12 weeks and daily use of 2.5- and 5-mg tadalafil for 24 weeks was well-tolerated and significantly improved erectile function (Porst et al., 2006; Rajfer et al., 2007), also in diabetic patients (Hatzichristou et al., 2008).

There is convincing evidence that tadalafil is efficacious in a number of special populations of men in which ED is a consequence of, for example, diabetes, radical prostatectomy, external beam radiotherapy for prostatic cancer, spinal cord injury, and lower urinary tract symptoms (Eardley et al., 2010). Side effects with tadalafil, most notably headache, flushing, indigestion, nasal congestion, and back or girdle pain may occur, but providing that the drug is used in line with the labeling recommendations, there is no convincing evidence in the literature of any significant safety issue, including CV, visual, and aural safety.

c. Vardenafil. Vardenafil is efficacious in the treatment of ED in the broad population at doses of 10 and 20 mg taken in an on-demand fashion (see, e.g., Morales et al., 2009; Eardley et al., 2010). Vardenafil is efficacious in a number of special populations of men in whom the ED is secondary to, for example, diabetes, radical retropubic prostatectomy, depression, hypertension, spinal cord injury, and hyperlipidemia. Vardenafil is also effective in men who have previously failed to respond to sildenafil (Morales et al., 2009; Eardley et al., 2010). Side effects with vardenafil, most often headache, flushing, indigestion, and nasal congestion, may occur, but providing that the drug is used in line with the labeling recommendations, there is no convincing evidence in the literature of any significant safety issue, including CV, visual, and aural safety (Morales et al., 2009; Eardley et al., 2010).

As mentioned previously, several other selective PDE5 inhibitors are in development (Hatzimouratidis and Hatzichristou, 2008; Eardley et al., 2010; Palit and Eardley, 2010). Because they all represent the same principal mode of action, differences in effect profile (i.e., time of onset, duration, and adverse effects) may be attributed to differences in selectivity and in pharmacokinetic properties.

d. Udenafil. Udenafil (Zydena) is a potent, selective PDE5 inhibitor claimed to also inhibit cGMP hydrolysis (Doh et al., 2002). Its pharmacokinetic profile includes a T_{max} of 1.0 to 1.5 h and a $t_{1/2}$ of 11 to 13 h (Kim et al., 2008). Udenafil is metabolized by CYP3A4, and the systemic exposure of the drug increased significantly when it was administered together with ketoconazole (Shin et al., 2010). In a broad population of Korean men with ED, Paick et al. (2008) showed that udenafil was effective. The effect of a 100-mg dose had a duration of at least 12 h (Park et al., 2010b). The drug was effective and safe in patients treated with antihypertensive medications (Paick et al., 2009) and given together with an α_1-AR antagonist to patients with lower urinary tract symptoms and ED, it improved both conditions without safety concerns (Chung et al., 2009a). The most common ad-

verse events reported have been facial flushing, nasal congestion, ocular hyperemia, and headache.

e. Mirodenafil. Mirodenafil, a pyrrolopyrimidinone compound, is a potent, reversible, and selective oral PDE5 inhibitor. It has been available in Korea since 2007. Preclinical studies revealed that the mirodenafil's selectivity toward PDE5 was 10-fold higher than that of sildenafil, whereas its inhibitory effects on other PDEs are much lower than that of sildenafil. The T_{max} and $t_{1/2}$ of mirodenafil were shown to be 1.25 and 2.5 h, respectively, and the coadministration of ketoconazole and rifampicin resulted in significant changes in systemic exposure to mirodenafil (Shin et al., 2009), confirming metabolism via CYP3A4 (Lee et al., 2008). Results from a phase 2 clinical study (Paick et al., 2008a) and a phase 3 clinical study (Paick et al., 2008b) provided evidence, for the efficacy and safety. The optimal doses in terms of efficacy and safety were determined from these studies to be 50 and 100 mg. Mirodenafil was shown to be effective and safe in men with ED concomitantly taking antihypertensive medications (Paick et al., 2010). In 112 Korean men with ED and diabetes, Park et al. (2010a) showed in an RCT that mirodenafil was effective and well tolerated. The most common adverse events were facial flushing, headache, nausea, and eye redness.

f. Lodenafil carbonate. Lodenafil is a dimer formed by two lodenafil molecules linked by a carbonate bridge. After ingestion, the bridge is broken delivering the active compound lodenafil (Toque et al., 2008). Early clinical trials with healthy volunteers showed good tolerability and bioavailability and revealed a linear absorption profile and good tolerability for doses up to 160 mg. After oral intake of 160 mg under fasting condition, C_{max} was 157 ng/ml, T_{max} was 1.2 h, and $t_{1/2}$ was 2.4 h (Lucio et al., 2007). A phase II clinical trial (Glina et al., 2009) showed efficacy and safety at doses of 20, 40, and 80 mg, and a phase III trial in 350 men with ED confirmed a satisfactory efficacy-safety profile (Glina et al., 2010). Adverse reactions included rhinitis, headache, flushing, visual disorder, and dizziness.

g. Avanafil. Avanafil is a pyrimidine derivative rapidly absorbed and quickly eliminated after oral administration. A mean T_{max} was reached at 0.33 to 0.52 h after oral dosing and then declined with a mean apparent $t_{1/2}$ of 5.36 to 10.66 h. AUC and C_{max} were proportional to dose, and the mean accumulation index on day 7 after a single daily dose was 0.98; concomitant food intake decreased the C_{max} by 24% (Jung et al., 2010; Limin et al., 2010). Two phase III studies have been completed, both with a positive outcome (Limin et al., 2010). One of these was a randomized, double-blind, placebo-controlled efficacy and safety study that evaluated three doses of avanafil (50-, 100-, and 200-mg) in 646 men with a history of ED. The other was a 16-week randomized, double-blind, placebo-controlled study evaluating two doses of avanafil (100 and 200 mg) in 390 men with both diabetes and ED. Adverse events were generally consistent with the known pharmacology of PDE5 inhibitors, and the most commonly reported adverse events included headaches, flushing, nausea, back pain, fatigue, and muscle cramps. Most of these events were mild in severity and resolved without medical treatment.

h. SLx-2101. The available information on SLx-2101 is scarce and most of it is published in abstract form (Hatzimouratidis and Hatzichristou, 2008; Palit and Eardley, 2010), but a structure is provided in Francis et al. (2009). SLx-2101 is a selective, fast-onset PDE5 inhibitor that is converted to an active metabolite, SLx-2081 (Myatt and Eardley, 2008). T_{max} was found at 1 h for SLx-2101 and 2.8 h for SLx-2081, and $t_{1/2}$ was 8 to 13 h for SLx-2101 and 9 to 14 h for SLx-2081. The duration of action of the "combination" (i.e., SLx-2101 and SLx-2081) was approximately 48 h. SLx-2101 has shown high potency, with therapeutic concentrations sustained for more than 24 h (Sweetnam et al., 2006). A randomized, double-blind, single-dose study was performed in healthy volunteers, who received one of five doses: 5, 10, 20, 40, or 80 mg (Donabedian et al., 2006; Prince et al., 2006). Positive effects on penile rigidity were observed approximately 24 h after administration at doses of 20, 40, and 80 mg. No clinically significant adverse CV effects were recorded at these doses. The most frequent adverse event was headache, although occasional temporary and reversible visual changes were noted in participants treated with the highest dose.

2. Prostaglandin E_1. Although PDE5 inhibitor remains the most common initial therapy in men with erectile dysfunction, intraurethral alprostadil may be a reasonable treatment option for sildenafil nonresponders (Jaffe et al., 2004), particularly in men having undergone prior radical retropubic prostatectomy (McCullough, 2001; McCullough et al., 2010). Vasoactive agents can be administered topically to the urethral mucosa and can apparently be absorbed into the corpus spongiosum and transferred to the CC. Retrograde filling of the cavernosal bodies through the deep dorsal vein and its circumflex branches seems to be the most relevant way of drug transfer after intraurethral application of prostaglandin E_1 (Bschleipfer et al., 2004).

PGE_1 (alprostadil) and a PGE_1/prazosin combination was demonstrated to produce erections in a majority of patients with chronic organic ED (Peterson et al., 1998). In a prospective, multicenter, double-blind, placebo-controlled study on 68 patients with long-standing ED of primarily organic origin (Hellstrom et al., 1996), transurethrally administered alprostadil produced full enlargement of the penis in 75.4%, and 63.6% of the patients reported intercourse. The most common side effect was penile pain, experienced by 9.1 to 18.3% of the patients receiving alprostadil. There were no episodes of priapism. In another double-blind, placebo-controlled study on 1511 men with chronic ED from various organic causes, 64.9% had intercourse successfully when taking

transurethral alprostadil compared with 18.6% on placebo (Padma-Nathan et al., 1997). Again, the most common side effect was mild penile pain (10.8%).

For men who find intracavernosal injections problematic, the ease of intraurethral administration of alprostadil is an option (Nehra et al., 2002). As mentioned, intraurethral alprostadil may also be an option in men having undergone prior radical retropubic prostatectomy (McCullough, 2001; McCullough et al., 2010). Penile pain remains a problem in many patients.

3. Organic Nitrates. Nitroglycerin and other organic nitrates are believed to cause smooth muscle relaxation by stimulating soluble GC via enzymatic liberation of NO (Feelisch,1992); theoretically, this seems to be a logical way to improve erection in patients with ED. Both nitroglycerin and isosorbide nitrate were found to relax isolated strips of human CC (Heaton, 1989). The observation that topical application of nitroglycerin to the penis may lead to erection adequate for sexual intercourse (Talley and Crawley, 1985) stimulated several investigations on the efficacy of this potential mode of treatment of ED. Despite effectiveness in patients with ED, demonstrated in several placebo-controlled studies (Andersson, 2001), the effect of transdermal nitroglycerin is limited, and this treatment does not seem to be a viable alternative today. As underlined previously, organic nitrates are contraindicated in men taking PDE5 inhibitors.

4. K^+-Channel Openers. Several K^+-channel openers (pinacidil, cromakalim, lemakalim, and nicorandil) have been shown to be effective in causing relaxation of isolated cavernosal tissue from both animals and man and to produce erection when injected intracavernosally in monkeys and humans (Andersson, 2001). However, only minoxidil, an arteriolar vasodilator used as an antihypertensive agent in patients with severe hypertension, seems to have been tried as an oral treatment in man. The clinical experiences with the drug are limited (Andersson, 2001), and K^+-channel opening with the drug, even if it may work in some patients, has not been proven in controlled clinical trials to be a viable option in men with ED. No new developments have been reported during the last decade.

5. α-Adrenoceptor Antagonists.

a. Phentolamine. Early studies with oral phentolamine showed some success in patients with nonspecific erectile insufficiency (Gwinup, 1988; Zorgniotti, 1992, 1994; Zorgniotti and Lizza, 1994). Zorgniotti (1992) considered nonintracavernosal, "on-demand" administration of phentolamine a promising approach for treatment of ED. Becker et al. (1998) performed a double-blind placebo-controlled trial with oral phentolamine 20, 40, and 60 mg in patients with ED and a high likelihood of organogenic etiology and found the drug to be of benefit. There were no serious complications, but some circulatory side effects were seen after 60 mg. In the doses needed for enhancing erectile responses (20–40 mg), few adverse CV effects have been observed (Goldstein, 2000; Goldstein et al., 2001).

Goldstein (2000) and Goldstein et al. (2001) reviewed experiences with oral phentolamine in ED and reported the results of large multicenter, placebo-controlled pivotal phase III clinical trials. The mean change in the erectile function as estimated by erectile function scores was significantly higher after use of active drug (40 and 80 mg) compared with placebo. Three to four times as many patients receiving phentolamine reported being satisfied or very satisfied compared with those receiving placebo. At doses of 40 and 80 mg, respectively, 55 and 59% of men were able to achieve vaginal penetration with 51 and 53% achieving penetration on 75% of attempts. The correction of ED or improvement to a less severe category of dysfunction was experienced by 53% of men with the 80-mg dose and 40% with the 40-mg dose of phentolamine. All trends of response were the same regardless of any concomitant medication. There were no severe adverse events. The most common side effects observed were nasal congestion (10%), headache (3%), dizziness (3%), and tachycardia (3%). Goldstein (2000) and Goldstein et al. (2001) concluded that phentolamine is safe, well tolerated, and efficacious for the treatment of ED. During the last decade, however, the success of PDE5 inhibitors seems to have hampered the enthusiasm for phentolamine, and no new evidence has been presented showing that the drug is a competitive alternative to other oral treatments of ED.

b. Yohimbine. Yohimbine is a pharmacologically well characterized α_2-AR antagonist that has been used for over a century in the treatment of ED (Morales, 2000). The drug is relatively selective for α_2-ARs, and even if other actions have been demonstrated (Goldberg and Robertson, 1983), these can be shown only in concentrations that most probably cannot be obtained in man. The site of action of yohimbine as a proerectile agent is most probably not peripheral, because α_1 is the predominant subtype of α-ARs in penile erectile tissue (Andersson, 2001; Prieto, 2008) and because intracavernosal injection of another more potent α_2-AR antagonist, idazoxan, did not produce penile erection in man (Brindley, 1986). In normal healthy volunteers, Danjou et al. (1988) found that intravenous infusion of yohimbine had no erectogenic effects. This does not exclude that orally administered yohimbine may be effective. The plasma half-life of yohimbine was found to be 0.6 h (Owen et al., 1987), whereas the plasma NA-increasing effects of the drug lasted for 12 h (Galitzky et al., 1990). This discrepancy may be explained by the presence of an active metabolite (Owen et al., 1987).

The effects of yohimbine have been investigated in several controlled trials on patients with different types of ED, but the effect has been modest (see, Andersson, 2001). It cannot be excluded that orally administered yohimbine may have a beneficial effect in some patients with ED. However, as a consequence of the conflicting

results, it is not currently recommended in most guidelines for management of ED.

6. Opioid Receptor Antagonists. It is well documented that long-term injection of opioids can lead to decreased libido and ED (Parr, 1976; Crowley and Simpson, 1978; Mirin et al., 1980; Abs et al., 2000; Hallinan et al., 2008), possibly because of hypogonadotropic hypogonadism (Mirin et al., 1980; Abs et al., 2000; Hallinan et al., 2008; Vuong et al., 2010). Assuming that endogenous opioids may be involved in sexual dysfunction, opioid receptor antagonists have been suggested to be effective as a treatment (Fabbri et al., 1989; Billington et al., 1990). There are some positive clinical experiences with naltrexone, which together with its active metabolite 6-β-naltrexol are competitive antagonists at μ- (and κ-opioid) receptors. This may illustrate the proof of principle (Andersson, 2001), and it cannot be excluded that increased inhibition by opioid peptides may be a factor contributing to nonorganic erectile failure in some patients. In such patients, naltrexone therapy may be a useful therapeutic agent. However, well controlled studies confirming this are lacking, and there seems to have been no new developments during the last decade.

7. Apomorphine. Apomorphine, a dopamine receptor agonist that stimulates both dopamine D_1- and D_2- like receptors, has been shown to induce penile erection in rats (Mogilnicka and Klimek, 1977; Benassi-Benelli et al., 1979) as well as in healthy men (Lal et al., 1984) and in men with ED (Lal et al., 1987, 1989). L-DOPA may also stimulate erection in patients with Parkinson's disease (see, e.g., Vogel and Schiffter, 1983). It has been suggested that dopamine D_2 receptor stimulation may induce penile erection in rats, whereas activation of D_1 receptors have the opposite effect (Zarrindast et al., 1992). In rhesus monkeys, quinelorane, a dopamine D_2 receptor agonist produced penile erection (Pomerantz, 1991), favoring the view that D_2 receptor stimulation is important for this response. Apomorphine has a higher affinity for the D_2- than D_1-like receptors (Rampin et al., 2003). D_2 receptors in the PVN are believed to be the main site for the induction of erections in the rat (Chen et al., 1999). This may also be the case in man (Brien et al., 2002).

Heaton et al. (1995) reported that apomorphine, absorbed through the oral mucosa, will act as an erectogenic agent. This has been largely confirmed in RCTs, and there is evidence that apomorphine is efficacious in the treatment of erectile dysfunction in the broad ED population at doses of 2 and 3 mg taken sublingually in an on demand fashion (Dula et al., 2000, 2001; Heaton et al., 2002; Von Keitz et al., 2002). The tolerability of apomorphine at a dose of 2 and 3 mg has been well investigated (Fagan et al., 2001; Adams et al., 2002; Ralph et al., 2002). The most common side effects are nausea, headache, and dizziness, with small numbers of patients developing syncope. This latter side effect was particularly noted at doses higher than those licensed for use in Europe. Overall, providing that the drug is used in line with labeling, there seems to be no evidence of significant tolerability of safety issues (Eardley et al., 2010).

Although the drug has shown statistically significant benefit over a placebo in the phase II/III clinical trials, examination of the pooled data revealed a low net benefit ratio (i.e., active efficacy minus placebo efficacy) with figures between only 11 and 13% (Stief et al., 2002). This limited efficacy, especially in patients with organic ED, which was confirmed in several studies (Perimenis et al., 2004a,b; Strebel et al., 2004; Gontero et al., 2005), leads to the suggestion that the drug could be best suited for men with mild ED. Subsequent comparative *prospective studies* between apomorphine SL and sildenafil provided clear evidence that sildenafil is more effective than apomorphine, and the high preference rates were in favor of sildenafil (Porst et al., 2007; Afif-Abdo et al., 2008; Giammusso et al., 2008; Pavone et al., 2008). Because of the superiority of sildenafil, apomorphine SL never reached noteworthy acceptance.

8. Trazodone. Trazodone is an "atypical" antidepressive agent, chemically and pharmacologically distinct from other currently available antidepressants (Haria et al., 1994). The drug selectively inhibits central 5-HT uptake and increases the turnover of brain dopamine but does not prevent the peripheral reuptake of NA (Georgotas et al., 1982). Trazodone has also been demonstrated to block receptors for 5-HT and dopamine, whereas its major metabolite, *m*-CCP, has agonist activity at 5-HT_{2C} receptors (Monsma et al., 1993). This metabolite induces erection in rats and selectively increases the spontaneous firing rate of the cavernosal nerves (Steers and de Groat, 1989). The mode of action of trazodone in depression is not fully understood; it has a marked sedative action. Trazodone has a serum half-life of approximately 6 h and is extensively metabolized (Haria et al., 1994). Trazodone and its major metabolite were shown to have an α-AR blocking effect in isolated human cavernosal tissue (Blanco and Azadzoi, 1987; Saenz de Tejada et al., 1991). Krege et al. (2000) showed trazodone to have high to moderate affinity for human α_1- and α_2-ARs, respectively, and that the drug did not discriminate between subtypes of α_1- and α_2-ARs. The active metabolite, *m*-CCP, seemed to have no significant peripheral effects.

Orally administered trazodone has been associated with priapism in potent men (Azadzoi et al., 1990) and with increased nocturnal erectile activity in healthy volunteers (Saenz de Tejada et al., 1991). Positive clinical experience with the drug has been reported (Lance et al., 1995). However, in double-blind, placebo-controlled trials in patients with different etiology of their ED, no effect of trazodone (150–200 mg/day) could be demonstrated (Meinhardt et al., 1997; Enzlin et al., 2000).

Even if the information from randomized, controlled clinical trials does not support the view that trazodone is

an effective treatment for most men with ED, it cannot be excluded that the drug may be an alternative in some anxious or depressed men. In a pilot study, it was observed that trazodone may be beneficial in the management of selective serotonin-reuptake inhibitor-induced sexual dysfunction (Stryjer et al., 2009). In men with ED and a degree of psychogenic component sufficient to reduce the efficacy of medical management, a combination of trazodone with sildenafil gave promising results in a pilot study (Taneja, 2007).

9. Melanocortin Receptor Agonists. MT-II is a synthetic cyclic heptapeptide that was initially designed as an artificial tanning agent (King et al., 2007). It is a cyclic nonselective melanocortin receptor agonist that, when injected subcutaneously, was found to be a potent initiator of penile erection in men with nonorganic ED (Wessells et al., 1998, 2000). However, yawning/stretching and in some cases severe nausea and vomiting limited its use.

PT-141 (bremelanotide) is a synthetic heptapeptide; it is a deaminated derivative and likely metabolite of MT-II. This compound has strong binding to MC receptors 1, 3, and 4, with a higher affinity for the MC4 receptor over MC3, where it acts as an agonist (Giuliano, 2004; King et al., 2007).

A number of clinical studies have evaluated the effect of PT-141 (Molinoff et al., 2003; Diamond et al., 2004, 2005; Rosen et al., 2004). PT-141 was administered intranasally at doses ranging from 4 to 20 mg to 32 healthy subjects in a randomized, double-blind, placebo-controlled crossover study (Diamond et al., 2004). This study was also carried out without visual sexual stimulation. Compared with placebo-treated subjects, PT-141 significantly increased erectile activity. The duration of erections with rigidity (Rigi-Scan monitoring) greater than 60% base was approximately 140 min in the subjects treated with 20 mg of PT-141 compared with 21 min in the placebo-treated group.

In a placebo-controlled crossover trial of 24 men with mild to moderate ED (Diamond et al., 2004), the effect of PT-141 (20 mg) was given together with visual sexual stimulation (erotic films). A 3-fold increase in erectile activity was observed in subjects given PT-141 compared with placebo. The duration of erection and penile rigidity were also significantly increased after PT-141 administration.

A randomized, prospective placebo-controlled crossover study compared treatment of 19 patients with ED with sildenafil (25 mg) alone versus sildenafil (25) with 7.5 mg of intranasal PT-141 (Diamond et al., 2005). Coadministration of the two agents resulted in significantly prolonged time of increased base rigidity (>60%) compared with sildenafil alone during a 2.5-h monitoring session. The combination of drugs was well tolerated with no significantly increased side effects over either sildenafil or PT-141 alone. No serious side effects have been reported after PT-141 administration in either normal subjects or in patients with ED.

It is obvious that MC receptor agonists may have effects in patients with ED that can be clinically useful. However, the relation between efficacy and adverse effects has to been determined in large RCTs before regulatory approval and possible clinical introduction.

D. Drugs for Intracavernosal Administration

Patients not responding to oral drugs may be offered intracavernosal injections. Among the many drugs tested (for reviews, see, e.g., Andersson, 2001; Hatzimouratidis and Hatzichristou, 2008; Eardley et al., 2010), only four, used alone or in combination, have become widely accepted and administered on a long-term basis: papaverine, phentolamine, PGE_1 (alprostadil), and VIP. The experimental and clinical experiences with some other agents, used for treatment and discussed below, are limited.

1. Papaverine. Intracavernosal papaverine injection was the first clinically effective pharmacological therapy for ED (Virag, 1982). The drug is often classified as a phosphodiesterase inhibitor, but it has a very complex mode of action and may be regarded as a "multilevel acting drug" (Andersson, 1994). It is difficult to establish which of its several possible mechanisms of action predominates at the high concentrations that can be expected when the drug is injected intracavernosally. In vitro, it has been shown that papaverine relaxes the penile arteries, the cavernosal sinusoids, and the penile veins (Kirkeby et al., 1990). In dogs, Juenemann et al. (1986) demonstrated that papaverine had a dual hemodynamic effect, decreasing the resistance to arterial inflow and increasing the resistance to venous outflow. The latter effect, which has been demonstrated also in man (Delcour et al., 1987), may be related to activation by papaverine of a veno-occlusive mechanism. Papaverine is effective but is no longer used as monotherapy because of its high rates of fibrosis and priapism.

2. α-Adrenoceptor Antagonists. Because NA is considered to be one of the main factors maintaining CC smooth muscle tone by stimulating α-ARs, it could be expected that blocking these receptors would cause an erectile response. However, the experiences α-adrenoceptor antagonists treatment as monotherapy have not been very successful.

a. Phentolamine. As mentioned previously, phentolamine is a competitive α-AR antagonist with similar affinity for α_1- and α_2-ARs, and this is considered its main mechanism of action. However, the drug can block receptors for 5-HT and cause release of histamine from mast cells. Phentolamine also seems to have another action, possibly involving NOS activation (Traish et al., 1997,1998). Phentolamine nonselectively blocks α-ARs, so it could be expected that by blocking prejunctional α_2-ARs, it would increase the NA release from adrenergic nerves, thus counteracting its own postjunctional

α_1-AR blocking actions. Whether or not such an action contributes to the limited efficacy of intracavernosally administered phentolamine to produce erection, is not known.

In dogs, phentolamine, like papaverine, decreased the resistance to arterial inflow to the penis. However, papaverine, but not phentolamine, increased the resistance to venous outflow (Juenemann et al., 1986). Lack of effect on venous outflow by intracavernosal phentolamine has also been demonstrated in humans (Wespes et al., 1989).

There is a general lack of information about the pharmacokinetics of phentolamine. The drug has a reduced efficacy when given orally, probably because of extensive first-pass metabolism. A discrepancy between the plasma half-life (30 min) and effect duration (2.5–4 h) has been demonstrated (Imhof et al., 1975); whether this can be attributed to active metabolites is not known. When the drug is given intracavernosally, the serum concentration of phentolamine will reach a maximum within 20 to 30 min, and then rapidly decline to undetectable levels (Hakenberg et al., 1990).

The most common side effects of phentolamine after intravenous administration are orthostatic hypotension and tachycardia. Cardiac arrhythmias and myocardial infarction have been reported, but these are very rare events. In theory, such effects may be encountered also after intracorporal administration, but so far this does not seem to be the case. Because a single intracavernosal phentolamine injection does not result in a satisfactory erectile response in most cases, the drug is widely used in combination with papaverine (Eardley et al., 2010) or with VIP (Dinsmore and Wyllie, 2008).

b. Thymoxamine. Thymoxamine (moxisylyte) has a competitive and relatively selective blocking action on α_1-ARs. In addition, it may have antihistaminic actions. Little is known about its pharmacokinetics, but after systemic administration, it has an effect duration of 3 to 4 h. Moxisylyte is a prodrug, rapidly transformed into an active metabolite in plasma (deacetylmoxisylyte). Urine is the main route of excretion (Marquer and Bressolle, 1998).

Moxisylyte was shown to produce erection when injected intracavernosally (Brindley, 1986), and in a double-blind crossover study, Buvat et al. (1989) showed it to be more active than saline but less active than papaverine. Buvat et al. (1989) reported on the experiences of intracavernosal injections of moxisylyte in 170 patients with ED and pointed out that the drug did not initiate erection but facilitated it by inducing prolonged tumescence. They also stressed that the main advantage of the drug was its safety. Only 2 of the 170 patients injected had prolonged erections. Buvat et al. (1991), comparing papaverine and moxisylyte, also found that moxisylyte had less tendency to produce corporal fibrosis than papaverine (1.3 versus 32%). The positive safety aspects were underlined by Arvis et al. (1996). In a comparative study between moxisylyte and PGE_1, Buvat et al. (1996) showed that PGE_1 was significantly more effective than moxisylyte (71 versus 50% responders), especially in patients with arteriogenic dysfunction (96 versus 46%). However, moxisylyte was significantly better tolerated than PGE_1, causing fewer prolonged erections and fewer painful reactions. As a facilitating drug, moxisylyte may be a reasonable alternative for treatment of ED. There seems to be no further development of the drug, and it is no longer used as a therapeutic alternative.

3. Prostaglandin E_1 (Alprostadil). PGE_1, injected intracavernosally, alone or in combination, is today a second-line treatment for ED (Alexandre et al., 2007; Albersen et al., 2011). Several aspects of its effects and clinical use have been reviewed previously (Linet and Ogrinc, 1996; Porst, 1996; Andersson, 2001; Alexandre et al., 2007). In clinical trials, 40 to 70% of patients with ED respond to intracavernosal injection of PGE_1. The reason why many patients do not respond is not known. Angulo et al. (2000) demonstrated that the combination of PGE_1 with *S*-nitrosoglutathione consistently relaxed penile smooth muscle whether or not it relaxed well to PGE_1. They suggested that the clinical response to PGE_1 may be limited in some patients by the specific lack of response of penile smooth muscle to PGE_1 while maintaining the ability to relax in response to agents that activate alternative relaxant pathways. A combination of PGE_1 and *S*-nitrosoglutathione had a synergistic interaction to relax penile trabecular smooth muscle, and it was speculated that such a combination may have significant therapeutic advantages in the treatment of male ED. However, there seem to have been no new developments of this combination.

PGE_1 is metabolized in penile tissue to PHE_0 (Hatzinger et al., 1995), which is biologically active and may contribute to the effect of PGE_1. PGE_1 may act partly by inhibiting the release of NA (Molderings et al., 1992), but the main action of PGE_1 and PGE_0 is probably to increase the intracellular concentrations of cAMP in the CC smooth muscle cells through EP receptor stimulation (Palmer et al., 1994; Lin et al., 1995).

PGE_1 is known to have a variety of pharmacological effects. For instance, it produces systemic vasodilation, prevents platelet aggregation, and stimulates intestinal activity. Administered systemically, the drug has been used clinically to a limited extent. Little is known about its pharmacokinetics, but it has a short duration of action and is extensively metabolized. As much as 70% may be metabolized in one pass through the lungs (Golub et al., 1975), which may partly explain why it seldom causes circulatory side effects when injected intracavernosally.

4. Vasoactive Intestinal Polypeptide. As discussed previously, a role for VIP as neurotransmitter and/or neuromodulator in the penis has been postulated by several investigators, but its physiological importance for penile erection has not been established (Andersson,

2001). However, the inability of VIP to produce erection when injected intracavernosally in potent men (Wagner and Gerstenberg, 1988) or men with ED (Adaikan et al., 1986; Kiely et al., 1989; Roy et al., 1990) indicated that it cannot be the main NANC mediator for relaxation of penile erectile tissues.

VIP has been shown to produce a wide range of effects. It is a potent vasodilator, inhibits contractile activity in many types of smooth muscle, stimulates cardiac contractility, and many exocrine secretions. It stimulates adenylyl cyclase and the formation of cAMP (Palmer et al., 1994; Fahrenkrug, 2001). VIP given intravenously can produce hypotension, tachycardia, and flushing (Frase et al., 1987; Krejs, 1988). However, the plasma half-life of the peptide is short, which may contribute to the fact that systemic side effects are rare when it is administered intracavernosally (McMahon, 1996; Dinsmore et al., 1999; Sandhu et al., 1999).

As mentioned, Wagner and Gerstenberg (1988) showed that VIP, even in a high dose (60 μg), was unable to induce erection on intracavernosal injection in potent men. On the other hand, when used in conjunction with visual or vibratory stimulation, intracavernosal VIP facilitated normal erection. Kiely et al. (1989) injected VIP, papaverine, and combinations of these drugs with phentolamine intracorporally in 12 men with ED of various causes. They confirmed that VIP alone is poor at inducing human penile erections. However, in combination with papaverine, VIP produced penile rigidity similar to that obtained with papaverine and phentolamine. Gerstenberg et al. (1992) administered VIP together with phentolamine intracavernosally to 52 patients with erectile failure. Forty percent of the patients had previously received treatment with papaverine alone or with papaverine together with phentolamine. After sexual stimulation, all patients obtained erection sufficient for penetration. Those patients previously treated with papaverine or papaverine/phentolamine stated that the action of the VIP combination was more like the normal coital cycle. No patient developed priapism, corporal fibrosis, or any other serious complication (Gerstenberg et al., 1992). These positive results have been confirmed by other investigators (McMahon, 1996; Dinsmore and Alderdice, 1998; Dinsmore et al., 1999; Sandhu et al., 1999; Dinsmore and Wyllie, 2008). Thus, Sandhu et al. (1999) found in a prospective, double-blind, placebo-controlled study on 304 patients with psychogenic ED, using a novel auto-injector, that more than 81% of patients and 76% of partners reported an improved quality of life. Similar results were obtained by Dinsmore et al. (1999). On the basis of these clinical trials, both the efficacy and safety of the combination has been confirmed, and the combination has approved for treatment of men with ED in the United Kingdom, Denmark, and New Zealand. Most commonly observed adverse effects were facial flushing and headache.

5. Apomorphine. In a placebo-controlled, double-blind study on healthy volunteers, it was shown that apomorphine, injected subcutaneously (0.25–0.75 mg), was able to induce erection (Lal et al., 1984). This was confirmed by Danjou et al. (1988), showing that apomorphine induced erection and potentiated the erection induced by visual erotic stimulation. There was no increase in libido, which was in agreement with previous observations (Julien and Over, 1984). In 28 patients with ED, Lal et al. (1989) found that 17 responded with erection after subcutaneous apomorphine (0.25–1.0 g); no erection developed after placebo. Segraves et al. (1991) also administered apomorphine subcutaneously (0.25–1.0 g) to 12 men with psychogenic ED in a double-blind and placebo-controlled study. They found a dose-related increase in maximal penile circumference. An erection with a >1-cm increase in maximal penile circumference was obtained in 11 of the 12 patients.

It cannot be excluded that a subgroup of impotent patients may have an impairment of central dopaminergic functions and that the principle of dopamine receptor stimulation can be used not only diagnostically but also therapeutically. The therapeutic potential of subcutaneous apomorphine, however, seems to be limited mainly because of frequently occurring side effects. High doses (i.e., up to 5–6 mg in adult patients) may cause respiratory depression, and in the low dose range (0.25–0.75 mg) where effects on penile erection can be demonstrated, emesis, yawning, drowsiness, transient nausea, lacrimation, flushing, and dizziness may occur (Lal et al., 1984; Segraves et al., 1991). Lal et al. (1987) observed that nonresponders, but not responders, experienced side effects. However, apomorphine administered subcutaneously does not seem to have an acceptable effect/side effect ratio, and it is no longer used therapeutically.

6. Linsidomine Chlorhydrate and Other NO Donors. Linsidomine, the active metabolite of the antianginal drug molsidomine, is believed to act by nonenzymatic liberation of NO (Feelisch, 1992). The pharmacology of linsidomine made it an interesting alternative for intracavernosal treatment of ED and preliminary studies seemed promising. However, the initial positive results were not confirmed (Andersson, 2001), and the drug is no longer used therapeutically.

Intracavernosal NO donors such as SNP seem effective to treat ED but have been controversial because of hypotensive side effects (Martinez-Piñeiro et al., 1995; Martínez-Piñeiro et al., 1998; Shamloul et al., 2005). Lasker et al. (2010) showed in the rat that sodium nitrite ($NaNO_2$), administered intracavernosally, increased ICP, decreased systemic arterial pressure, and was 1000-fold less potent than the NO donor SNP. They suggested that in the rat, $NaNO_2$ is converted to vasoactive NO in the corpora cavernosum and systemic vascular bed by different mechanisms. Thus, experiments with the NOS inhibitor L-NAME and the xanthine oxidoreductase inhibitor allopurinol sug-

gested that nitrite bioactivation in the corpora was mediated through eNOS, whereas nitrite bioactivation in the systemic vascular beds was due largely to the activity of xanthine oxidoreductase. It was also suggested that the ability of nitrite to enhance erectile activity motivates further investigation in the use of nitrite as a therapeutic agent for ED.

7. Combination Therapy. Phentolamine, papaverine, PGE_1, and VIP are the vasoactive agents most commonly used in combination therapy to treat ED. In theory, combination therapy may offer better efficacy, because many of the drugs would be assumed to act synergistically but a reduction in incidence of side effects and cost per dose can also be expected. An often-used combination is trimix, a mixture of papaverine, phentolamine, and PGE_1. Bechara et al. (1996) reported better results with the combination than with PGE_1 alone. However, Seyam et al. (2005) compared trimix using a 1-mg dose of phentolamine and different doses of papaverine and PGE_1 with a 20-μg dose of PGE1, found no significant differences in hemodynamic effects, rigidity, pain, and self-satisfaction between the two drugs. However, trimix produced a longer duration of erection and more priapism than PGE_1. This and most other combination therapies remain unlicensed. However, the combination of VIP and phentolamine has been approved in several countries.

E. Gene Therapy

Few, if any of the available pharmacological treatments for ED will improve the underlying causes of the disorder or "cure" the disease. Efforts have therefore been directed to the development of gene- and cell-based approaches to correct the molecular and tissue defects responsible for ED. In many ways, the penis is a good target tissue for gene therapy because of its physical location, low blood flow in the nonerect state, and the internal structure of the CC. Gene therapy for treatment of ED has been reviewed extensively and proposed as one of potential new therapies for ED associated with, e.g., aging, diabetes, and cavernosal nerve injury (Melman et al., 2009; Burnett et al., 2010; Harraz et al., 2010; Melman and Davies, 2010; Yoshimura et al., 2010). Almost all studies have been made in animals, and so far, only one has been performed in humans. Considering their importance for the erectile process, genes involved in the nitrergic pathway, such as *NOS*, have been tested extensively. For the neurogenic type of ED induced by diabetes or cavernosal nerve injury, genes encoding different types of neurotrophic factors, which can enhance nerve regeneration, have been proposed. K^+ channel genes, which functionally enhance relaxation of cavernosal smooth muscle, have also been tested. Because gene therapy involves the transfer of genetic material to a target cell or tissue, both viral and nonviral methods have been used, the latter including the introduction of naked DNA or plasmid DNA (Christ and Melman, 1998). Using this method, the efficacy of intracavernosal gene transfer of naked hSlo cDNA that encodes the human BK channel α-subunit was investigated with positive results in aged or diabetic rats and in male cynomolgus monkeys with ED secondary to diet-induced atherosclerosis (Christ et al., 1998, 2004, 2009; Melman et al., 2003, 2008). A phase I safety clinical trial in men with ED using the plasmid containing hSlo cDNA has been completed (Melman et al., 2006). The results were encouraging from a safety point of view, and two of the men participating in the study responded with improved erections for 6 months after transfer. Despite these promising initial results, the development has been slow, and no further studies have been reported.

F. Angiogenesis Therapy

The potential for the use of angiogenic factors to restore erectile function, either without the need for PDE5 inhibitors or by enhancing the effect of this class of agents has attracted great interest (Lysiak et al., 2010). Vascular endothelial growth factor is expressed in both rat and human CC (Burchardt et al., 1999a,b), and the expression is down-regulated in the CC of, e.g., hypercholesterolemic rats and rabbits (Byrne et al., 2001; Xie et al., 2005; Ryu et al., 2006). Numerous studies in several animal models of ED have successfully employed intracavernosal delivery of vascular endothelial growth factor and other angiogenic factors (Lysiak et al., 2010). The observations made suggest an advantageous role for therapeutic angiogenesis in the treatment, if not the prevention, of vasculogenic ED. However, human investigations have not yet begun (Lysiak et al., 2010).

G. Revascularization of the Internal Pudendal Artery

There has been a renewed interest in the role of changes in the internal pudendal artery (IPA) in the pathophysiology of ED, both preclinically (Hale et al., 2009; Hannan et al., 2010) and clinically (Hale et al., 2009; Rogers et al., 2010). There is a similarity between atherosclerotic changes in this vessel and the coronary arteries (Rogers et al., 2010), and the use of drug-eluting stents, similar to those used in the coronary arteries, have been suggested to restore blood flow in patients with ED and stenosed IPA. Studies with stents releasing the antiproliferative agent zotarolimus are currently ongoing to investigate the safety, feasibility, and appropriate patient selection for percutaneous revascularization of IPA stenoses in men with ED (Rogers et al., 2010). The results of these studies will decide the possible future and place of this approach in the treatment of ED.

V. Conclusions and Future Perspectives

The most successful approach to treat ED has been drugs aiming at mechanisms in the target organ. PDE5 inhibitors have had a tremendous impact on the treatment of ED, but are not always effective (e.g., in patients with diabetes). Despite significant progress, the differ-

ent steps involved in neurotransmission, impulse propagation, and intracellular transduction of neural signals in penile smooth muscles need further investigation. It should be remembered that most of the pharmacological options for ED treatment do not influence the progress of the underlying pathophysiology and do not cure the disease. This means that other approaches such as gene- or cell-based therapies may be future directions for research. Increased knowledge of changes in penile tissues associated with ED may lead to increased understanding of pathogenetic mechanisms and to prevention of the disorder. The possibility of using drug-eluting stents in patients with stenosed IPA is exciting and may open up for future preclinical and clinical research focusing on the molecular biology of IPA in disease states and on clinical applicability of this approach.

The fact that CNS mechanisms play an important role for erection and as targets for ED drugs has been recognized, but drugs aiming at CNS targets have so far not been very successful. The supraspinal and spinal regulation of the erectile process involves several transmitters, including dopamine, serotonin, NA, NO, and peptides, such as oxytocin and adrenocorticotropin/α-MSH, but is still only partly known. Detailed knowledge of these systems will be important for the discovery of novel pharmacological agents for the treatment of ED. Because erection is only one (albeit important) factor of the male sexual response cycle, the promise of CNS-active drugs is that they may also affect other components (desire-arousal-excitement-orgasm) in a positive way. Further research to prove this is desirable.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: Andersson.

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