

The Sexiest Channels Alive:

The Role of Ion Channels in Penile Erection

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Penile erection is a spinal reflex that involves integration of tactile, olfactory, visual and auditory inputs in the central nervous system (CNS). Despite the involvement of CNS higher functions, penile erection is, in the end, the result of the relaxation of the specialized smooth muscle cells of the penis. Relaxation of the smooth muscle cells permits increased blood flow into the penis that provides the rigidity needed for erection. The coordinated relaxation of the penile smooth muscle cells is achieved by the synchronized function of several surface receptors, ion channels, intracellular transduction mediators and contractile proteins whose molecular identity and regulation has become increasingly recognized. This article will briefly review the known facts about smooth muscle regulation in penile erection with a special emphasis on ion channels.

Molecular Regulation of Penile Erection

The anatomy of the penis consists of endothelial-lined sinusoidal structures surrounded by a specialized type of smooth muscle cell called corporal smooth muscle. In the flaccid state, the corporal smooth muscle cells keep a high muscle tone (are in a contracted state) preventing entry of blood into the corporal tissue. When an erection is initiated, the corporal smooth muscle cells relax, allowing massive influx of blood into the sinusoidal structures, thus providing the necessary rigidity of the organ.

The centrality of the corporal myocyte function in penile erection and sexual function is underscored by the fact that more than 80% of patients with erectile dysfunction can be treated by methods that induce smooth muscle relaxation of the penis.

From the molecular point of view, the intracellular Ca^{2+} concentration is the key regulator of smooth muscle tone. Elevated levels of intracellular Ca^{2+} induce myosin protein phosphorylation (via the myosin light chain kinase (MLCK)) and subsequent muscle contraction. Thus, corporal

smooth muscle relaxation and penis erection are the end result of mechanisms that lower the intracellular Ca^{2+} concentration.^{1,2}

The delicate balance of intracellular Ca^{2+} concentration is tightly regulated by several ion channels and intracellular mediators and will be discussed in some detail below. For a simplified illustration of the molecular mechanisms governing corporal smooth muscle contraction and relaxation see Figure 1.

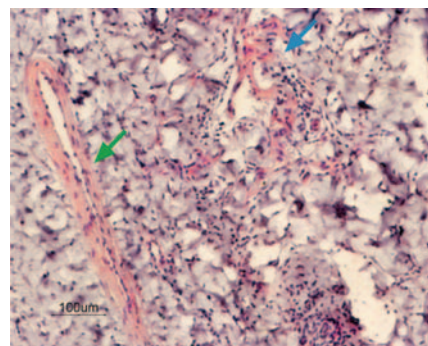
1. Keeping the Intracellular Ca^{2+} High and the Penis Down (Flaccid State)

High intracellular Ca^{2+} concentration is achieved by two means: release of Ca^{2+} from the sarcoplasmic reticulum (the intracellular compartment for Ca^{2+} storage) and entry of Ca^{2+} through the plasma membrane.

Release of Ca^{2+} from the sarcoplasmic reticulum (SR) is achieved through the opening of ligand-gated IP_3 receptors (IP_3R) present in the SR membrane. Levels of the ubiquitous second messenger IP_3 are increased after phospholipase

C (PLC) hydrolyzes phosphatidylinositol 4, 5-biphosphate (PIP_2) to 1,2-diaclylglycerol (DAG) (which activates protein kinase C) and IP_3 .^{1,2} PLC in turn is activated by receptors coupled to GTP binding proteins or G-protein coupled receptors (GPCRs). The best characterized GPCRs involved in inducing high intracellular Ca^{2+} (and therefore

Expression of K_{Ca} 1.1 in Rat Penis



Transversal section of the rat penis was stained with Anti- K_{Ca} 1.1 antibody (#APC-107). Strong and specific immunostaining is evident in both corpus cavernosum smooth muscle cells (blue arrow) and in the muscular layer of the penis artery (green arrow). Universal Immuno-alkaline-phosphatase Polymer followed by New Fuchsin Substrate (Histofine, Nichirei Corp) was used for the color reaction. Counterstain is Hematoxilin.

maintaining the penis in a flaccid state) are the α -adrenoreceptor (activated by noradrenaline) and the ET_A receptor (activated by endothelin).

Corporal smooth muscle cells receive high adrenergic innervations, which release the neurotransmitter noradrenaline (NA) that is widely believed to be the main agent responsible for keeping the corporal myocyte in a contracted state and the penis in a relaxed one.¹ NA acts by binding to the α -adrenoreceptor family that consists of $\alpha 1$ ($\alpha 1_A$, $\alpha 1_B$, $\alpha 1_D$) and $\alpha 2$ ($\alpha 2_A$, $\alpha 2_B$, $\alpha 2_C$) subtypes. The exact receptor subtypes involved in corporal myocyte contraction is a matter of some debate but adrenergic receptors of the $\alpha 1$ subtype are believed to be the dominant ones.^{1,3}

Another agent believed to be important in maintaining high intracellular Ca^{2+} levels through the PLC/IP₃ pathway in the corporal smooth muscle are the endothelins (ET). ET belongs to a family of peptides that are synthesized mainly in endothelial cells as a precursor that is subsequently cleaved and released in its mature form. In corporal myocytes ET acts by binding to the ET_A subtype receptor that induces a rise in intracellular Ca^{2+} concentration leading to myocyte contraction.^{1,4}

In addition to the release of Ca^{2+} from the intracellular sarcoplasmic stores, keeping a high cytosolic Ca^{2+} concentration requires Ca^{2+} entry from the extracellular space. In corporal myocytes (as in other smooth muscle cell types) this is achieved mainly by the opening of L-type voltage-dependent Ca^{2+} channels situated in the plasma membrane. The L-type voltage-dependent Ca^{2+} channels (so called because of their long-lasting activation) include four members: $Ca_v 1.1$, $Ca_v 1.2$, $Ca_v 1.3$ and $Ca_v 1.4$. These channels open in response to a strong membrane depolarization and remain activated for relatively long periods of time, allowing the continuous influx of Ca^{2+} into the intracellular space. In addition, the channels can also be regulated by several signaling mechanisms including PKC-mediated phosphorylation (that enhances channel activity) and protein kinase G (PKG) (that inhibits channel function).^{2,5}

2. Keeping the Intracellular Ca^{2+} Down and the Penis Up (Erection)

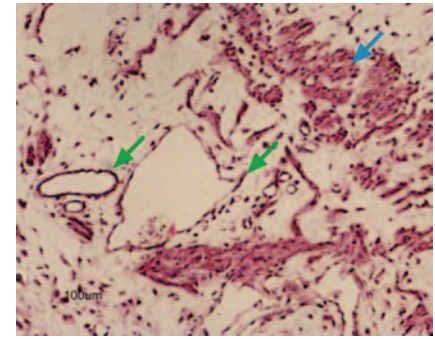
As stated above, penile erection is the end result of smooth muscle relaxation that can be initiated by sensory stimulation that activates the CNS (i.e following visual, auditory, etc.) pathways. The CNS activates peripheral nerves that innervates the penis and include cholinergic and nonadrenergic-noncholinergic (NANC) nerves among others.⁶

The best characterized relaxation-inducing agent is nitric oxide (NO) that is released from the NANC nerves (also called nitrenergic). NO can also be released by the endothelial cells surrounding the corporal smooth muscle cells. NO is a product of the processing of L-Arginine by the nitric oxide synthase (NOS) enzyme.⁷

NO diffuses into the corporal smooth muscle where it activates the soluble guanylyl cyclase (sGC) enzyme to produce the second messenger cGMP. cGMP in turn activates PKG which lowers the intracellular Ca^{2+} concentration by multiple mechanisms.

One of these mechanisms is the activation of the Ca^{2+} -dependent K^+ channel $K_{Ca} 1.1$ (also known as Maxi-K or BK_{Ca}). The $K_{Ca} 1.1$ is a member of a specialized type of K^+ channels that can be activated by both membrane depolarization and high intracellular Ca^{2+} . Activation of the channel produces an efflux of K^+ along its electrochemical gradient and a concomitant membrane hyperpolarization. This in turn closes the voltage-dependent L-type Ca^{2+} channels, effectively lowering the cytosolic Ca^{2+} concentration producing corporal smooth muscle relaxation and penile erection.^{8,9} As mentioned above, PKG may also act directly on voltage-dependent Ca^{2+} channels to close the channels and terminate Ca^{2+}

Expression of $K_{Ca} 1.1$ in Pregnant Rat Uterus

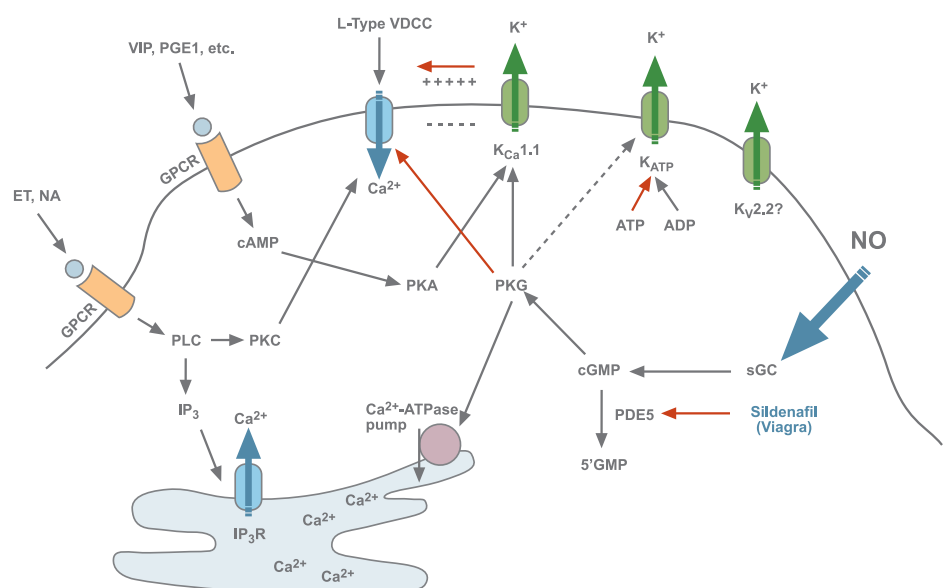


Longitudinal section of the miometrium in pregnant rat uterus stained with Anti- $K_{Ca} 1.1$ antibody (#APC-105) (1:50). Strong and specific staining is evident in smooth muscle cells both in the miometrium (blue arrow) and muscular layers of blood vessels (green arrows). Peroxidase reaction with DAB were used for the color reaction. Counterstain is Hematoxylin.

entry into the cell. Another mechanism by which PKG lowers the cytosolic Ca^{2+} concentration is by activating the sarcoplasmic Ca^{2+} -ATPase pump that takes Ca^{2+} from the cytosol and into the luminal side of the sarcoplasmic reticulum.

The central role of the NO/cGMP/PKG signaling pathway in penile erection is demonstrated by

Fig. 1: Molecular Mechanisms Regulating Penile Erection



Penile erection is the end result of corporal smooth muscle relaxation. This is achieved by the coordinated actions of several ion channels, signaling mechanisms and receptors that produce a decrease in cytosolic Ca^{2+} levels. Gray arrows show positive interaction. Red arrows show negative interactions. Dashed arrows are putative interactions. Abbreviations: GPCR, G-protein coupled receptor; ET, endothelin; NA, noradrenaline; VIP, vasoactive intestinal peptide; PGE1, prostaglandin E1; L-type VDCC, L-type voltage dependent Ca^{2+} channel; sGC, soluble guanylyl cyclase; NO, nitric oxide; PDE5, phosphodiesterase 5.

examining the mechanism of action of sildenafil (Viagra, Pfizer Inc., New York), the popular drug for erectile dysfunction. Sildenafil is a phosphodiesterase (PDE) inhibitor, an enzyme that catalyzes degradation of cGMP. There are six subtypes of PDEs (PDE1-PDE6), each with its own tissue distribution and substrate specificity. PDE5, the specific target of sildenafil is highly expressed in corporal smooth muscle cells. Thus, inhibition of PDE5 keeps cGMP levels in the corporal myocytes elevated longer, therefore allowing a more pronounced cytosolic Ca^{2+} decrease and stronger erections.¹⁰

Similarly, the importance of the $K_{Ca}1.1$ channel in penile erection has also been demonstrated by the use of inhibitors such as the toxin **Charybdotoxin** or by examination of knockout mice lacking the $K_{Ca}1.1$ gene.^{11,12} In both cases corporal smooth muscles were unable to relax and therefore to maintain an erection. Conversely, openers of the $K_{Ca}1.1$ channel have been studied as drugs for erectile dysfunction. An orally available $K_{Ca}1.1$ opener (BMS-223131) was evaluated in this regard while more recently, a gene transfer therapy protocol with the $K_{Ca}1.1$ gene underwent Phase I clinical trials.^{9,13,14} In either case, the idea is that by increasing either the open probability or the expression of the $K_{Ca}1.1$ channel, smooth muscle membrane hyperpolarization and the concomitant decrease in cytosolic Ca^{2+} will be greater, therefore leading to more potent erections.

It is worth mentioning that increased expression of the $K_{Ca}1.1$ channel in corporal smooth muscle as a result of gene therapy won't cause per se relaxation of the smooth muscle and erections. Since the channel is essentially quiescent in the smooth muscle cells in the contracted state, only activation of the signaling pathway (i.e. through the NO/PKG pathway) will open the channel and produced the required membrane hyperpolarization and penile erection. Another advantage to the gene therapy approach is that its effect will last for a few months. This is in contrast to the effect of oral drugs such as sildenafil that must be used in advance and whose effect lasts only a few hours.¹⁴

It should be emphasized that the $K_{Ca}1.1$ channel contributes to corporal cell relaxation in response to other signaling pathways and not only to the NO-PKG one. For example, the prostanoid prostaglandin E1 (PGE1) has been extensively used as a therapeutic agent for the treatment of erectile dysfunction. PGE1 signals to the corporal smooth muscle cells via a GPCR that activates the enzyme adenylate cyclase and increases cAMP levels in the cell. cAMP in turn activates the protein kinase PKA that stimulates the $K_{Ca}1.1$ channel.¹⁵ Other agents known to induce corporal smooth muscle relaxation such as vasoactive intestinal peptide (VIP) and calcitonin gene-related peptide (CGRP) probably work along the

same pathway.^{1,6}

Besides the $K_{Ca}1.1$ channel other K^+ channels have been shown to play an important role in penile erection. The most physiologically relevant K^+ channel in addition to $K_{Ca}1.1$ is the K_{ATP} channel. The K_{ATP} channels are heteromultimers of two radically different subunits. The first component is the pore-forming subunit that is composed of members of the inward rectifier $K_{ir}6.x$ subfamily and includes two members: $K_{ir}6.1$ and $K_{ir}6.2$. The other component of the K_{ATP} channels is the sulfonylurea receptor (SUR) that is a member of the ATP-binding cassette (ABC) superfamily. There are three different isoforms of the SUR subunit SUR1, SUR2A and SUR2B, where the last two are alternative splice variants of the same gene.

K_{ATP} channels as their name suggests are K^+ channels that are highly sensitive to the ATP/ADP ratio in the cell. ATP closes the channel while ADP activates them. The molecular identity of the K_{ATP} channels in the corporal smooth muscle cells is not clear yet, however a channel composed of the $K_{ir}6.1$ and SUR2B subunits appears to be the likely candidate.^{1,8} The importance of the K_{ATP} channel in penile erection has been demonstrated by the use of K_{ATP} channel activators as erection-inducing agents. Indeed, several known K_{ATP} channel openers such as pinacidil, cromakalim and lemakalin have been demonstrated to induce corporal smooth muscle relaxations and erections *in vitro* (isolated tissue strips) and *in vivo* (when injected into the penis). The molecular regulation of the K_{ATP} channel in the corporal smooth muscle is not well understood, but a direct activation of the channel by PKG has been observed in other preparations.¹⁶

Finally, other K^+ channels such as $K_v2.2$ have been described as well in corporal smooth muscle but their physiological significance remains to be established.¹⁷

Conclusions

Penile erection is a complex process that involves the coordinated function of neural, vascular and smooth muscle cells in the penis. The harmonized function of these cellular types ultimately results in smooth muscle relaxation and therefore penile erection. Ion channels have a tremendous importance in these processes and have been identified as potential targets for the development of therapies for the treatment of erectile dysfunction.

Given that the most successful erectile dysfunction therapy today, the PDE5 inhibitor sildenafil (Viagra), is effective in only about 60% of men and has significant side effects, there is an increasing interest in developing better therapies. Ion channels could well be the new therapeutic frontier for penile erection in the 21st century.

References:

- Andersson, K.E. (2001) *Pharmacol. Rev.* **53**, 417.
- Christ G.J. and Hodges, S. (2006) *Br. J. Pharmacol.* **147**, S41.
- Traish, A. *et al.* (2000) *Int. J. Impot. Res.* **12** (Suppl. 1), S48.
- Granchi, S. *et al.* (2002) *Mol. Hum. Reprod.* **8**, 1053.
- Keef, K.D. *et al.* (2001) *Am. J. Physiol. Cell. Physiol.* **281**, C1743.
- Moreland, R.B. *et al.* (2001) *J. Pharmacol. Exp. Ther.* **296**, 225.
- Toda, N. and Okamura, T. (2003) *Pharmacol. Rev.* **55**, 271.
- Christ, G.J. (2000) *Int. J. Impot. Res.* **12** (Suppl. 4), S15.
- Christ, G.J. (2002) *J. Androl.* **23**, S10.
- Michelakis, E. *et al.* (2000) *Can. Med. Assoc. J.* **163**, 1171.
- Spektor, M. *et al.* (2002) *J. Urol.* **167**, 2628.
- Werner, M.E. *et al.* (2005) *J. Physiol.* **567**, 2, 545.
- Melman, A. *et al.* (2005) *Eur. Urol.* **48**, 314.
- Melman, A. (2006) *Int. J. Impot. Res.* **18**, 19.
- Lee, SW. *et al.* (1999) *Int. J. Impot. Res.* **11**, 189.
- Han, J. *et al.* (2001) *J. Biol. Chem.* **276**, 22140.
- Malysz, J. *et al.* (2002) *J. Androl.* **23**, 899.

Related Products

Compound	Product #
Ion Channel Antibodies	
Anti- $K_{Ca}1.1$ (1098-1196) (BK_{Ca})	APC-021
Anti- $K_{Ca}1.1$ (1184-1200) (BK_{Ca})	APC-107
Anti- $K_{ir}6.1$	APC-105
Anti- $K_{ir}6.2$	APC-020
Anti- $K_v2.2$	APC-120
Anti-IP ₃ R1	ACC-019
Anti- $Ca_v1.2$ (α_{1c})	ACC-003
Anti-Human $Ca_v1.2$ (α_{1c})	ACC-022
Anti- $Ca_v1.2a$ (α_{1c} Cardiac)	ACC-013
Anti- $Ca_v1.3$ (α_{1v})	ACC-005
G-Protein Coupled Receptors Antibodies	
Anti-ET-A	AER-001
Anti-ET-B	AER-002
L-Type Voltage-Gated Ca^{2+} Channel Blockers	
Calcicludine	C-650
Calciseptine	C-500
FS-2	F-700
TaiCatoxin	T-800
L-Type Voltage-Gated Ca^{2+} Channel Activator	
(s)-Bay K 8644	B-350
$K_{Ca}1.1$ Channel Blockers	
rCharybdotoxin	RTC-325
rliberiotoxin	RTI-400
rSlotoxin	RTS-410
$K_{Ca}1.1$ Channel Activators	
Isopimaric Acid	I-370
Pimaric Acid (PiMA)	P-270