

·Review·

Molecular Yin and Yang of erectile function and dysfunction

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Abstract

In regard to erectile function, Yin is flaccidity and Yang erection. In the past decade, research has mostly focused on the Yang aspect of erectile function. However, in recent years, the Yin side is attracting increasingly greater attention. This is due to the realization that penile flaccidity is no less important than penile erection and is actively maintained by mechanisms that play critical roles in certain types of erectile dysfunction (ED); for example, in diabetic patients. In addition, there is evidence that the Yin and Yang signaling pathways interact with each other during the transition from flaccidity to erection, and vice versa. As such, it is important that we view erectile function from not only the Yang but also the Yin side. The purpose of this article is to review recent advances in the understanding of the molecular mechanisms that regulate the Yin and Yang of the penis. Emphasis is given to the Rho kinase signaling pathway that regulates the Yin, and to the cyclic nucleotide signaling pathway that regulates the Yang. Discussion is organized in such a way so as to follow the signaling cascade, that is, beginning with the extracellular signaling molecules (e.g., norepinephrin and nitric oxide) and their receptors, converging onto the intracellular effectors (e.g., Rho kinase and protein kinase G), branching into secondary effectors, and finishing with contractile molecules and phosphodiesterases. Interactions between the Yin and Yang signaling pathways are discussed as well. (*Asian J Androl* 2008 May; 10: 433–440)

Keywords: erectile function; erectile dysfunction; molecular mechanisms; Rho kinase signaling; cyclic nucleotide signaling; Yin–Yang

1 Introduction

The Chinese Dau (Tao) philosophy stipulates that all things exist in two opposing yet complementary states,

Yin and Yang. Simply put, Yin is feminine, Yang masculine. And, when it comes to the penis, flaccidity is Yin and erection Yang. In ancient Egypt, an erect penis, depicted on wall carvings in several temples, symbolizes power, fertility, and victory (e.g., <http://www.touregypt.net/featurestories/min.htm>). In the modern day, most of us can still recall the phenomenon that swept across the globe with the debut of Viagra (Pfizer Inc., New York, USA). So, it can be safely assumed that having a strong Yang in the penis is a universal and timeless desire in all human races. However, the fact that every man's penis stays most of

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the time in the Yin state tells us loud and clear that, no matter how hard we try, Yin will always be the dominant force in penises. By recognizing this fact, hopefully we will all agree that, after decades of research focusing on the Yang side, we should now take a serious look at the process in which the Yin force operates in the penis. And, as the Yin–Yang philosophy further proclaims, a better understanding of the Yin should lead to a better management of the Yang.

So, what is the Yin force? Is it simply a static, sort of lifeless, state of the penis? Or is it something active and dynamic? Once again, just as the Yin–Yang philosophy dictates, whether it is Yin or Yang, there is always something going on within each state. We all know that the cavernous smooth muscle (CSM) is contracted when the penis is flaccid. So something must be in operation to maintain the contracted state of the CSM.

2 Yin and Yang of erectile function

Nature’s many activities – such as day and night, or the four seasons – go around and around like a spinning wheel of Tai Chi. So does the erection cycle (Figure 1), which is initiated by sexual stimulation and maintained during continuous sexual stimulation. Erection starts to

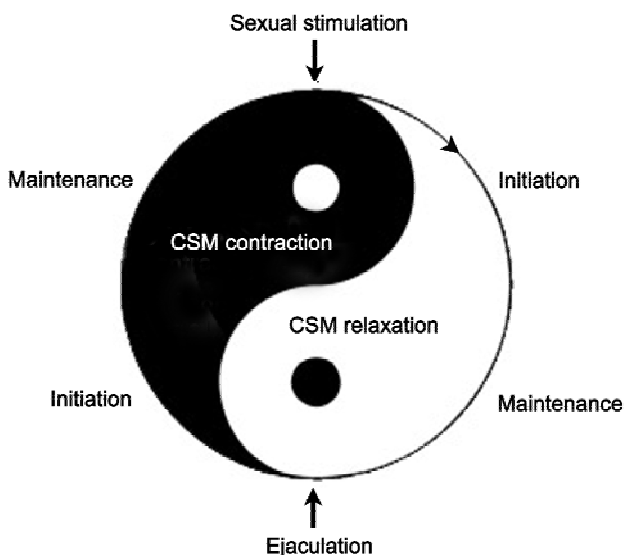


Figure 1. The erection cycle. Erection is initiated by sexual stimulation and maintained during continuous sexual stimulation. Erection starts to subside at ejaculation and the subsequent flaccidity is maintained until the next sexual stimulation. CSM, cavernous smooth muscle.

subside at ejaculation or at the cessation of sexual stimulation and the subsequent flaccidity state is maintained until the next sexual stimulation or nocturnal erection. Thus, both the erection and the flaccidity states of the penis exist in two phases, initiation and maintenance. In Figure 1, erection and flaccidity are equated to relaxation and contraction, respectively, of the CSM.

Initiation of contraction begins with the release of norepinephrin from sympathetic nerves [1]. Norepinephrin binds to adrenergic receptor in the cytoplasmic membrane of CSM cells (CSMC) [2–6]. The adrenergic receptor belongs to the G-protein-coupled receptor (GPCR) family and, in its guanosine triphosphate (GTP)-bound state, activates phospholipase C- β , which splits phosphatidylinositol (4,5)-bisphosphate into inositol trisphosphate (IP₃) and diacylglycerol. Binding of diacylglycerol to protein kinase C (PKC) could lead to CSM contraction [7]; however, the detail of this pathway is not well understood and interested readers are advised to read a recent review by Larsson [8]. In contrast, the other pathway that descends from IP₃ has been well characterized: binding of IP₃ to its receptor, IP₃R, in the sarcoplasmic reticulum triggers the release of calcium (Ca) from the sarcoplasmic reticulum; calcium binds to calmodulin (CaM), which then binds to and activates myosin light chain kinase (MLCK); MLCK phosphorylates myosin light chain (MLC), which then binds to and activates actin, resulting in contraction (Figure 2).

Three extracellular molecules, adrenalin [1], endothelin-1 [9–14], and angiotensin II [15–18], are primarily responsible for the maintenance of CSM contraction. Each of them binds to a different GPCR in the cytoplasmic membrane, leading to the activation of guanine exchange factor, which converts RhoA-guanosine diphosphate (GDP) to RhoA-GTP. RhoA-GTP dissociates from GDP dissociation inhibitor and migrates to the cytoplasmic membrane, where it binds to and activates Rho kinase (ROCK). ROCK phosphorylates and inactivates myosin light chain phosphatase (MLCP), allowing MLC to stay phosphorylated and consequently actin-contracted. This ROCK signaling pathway (Figure 3) is responsible for the maintenance of smooth muscle contraction [19].

The contracted state is disrupted by sexual stimulation, which triggers the release of nitric oxide (NO) from cavernous nerves in the penis (Figure 4) [20]. NO diffuses into CSMC and activates soluble guanyl cyclase [21], which then catalyzes the conversion of GTP to cyclic guanosine monophosphate (cGMP) [22]. cGMP acti-

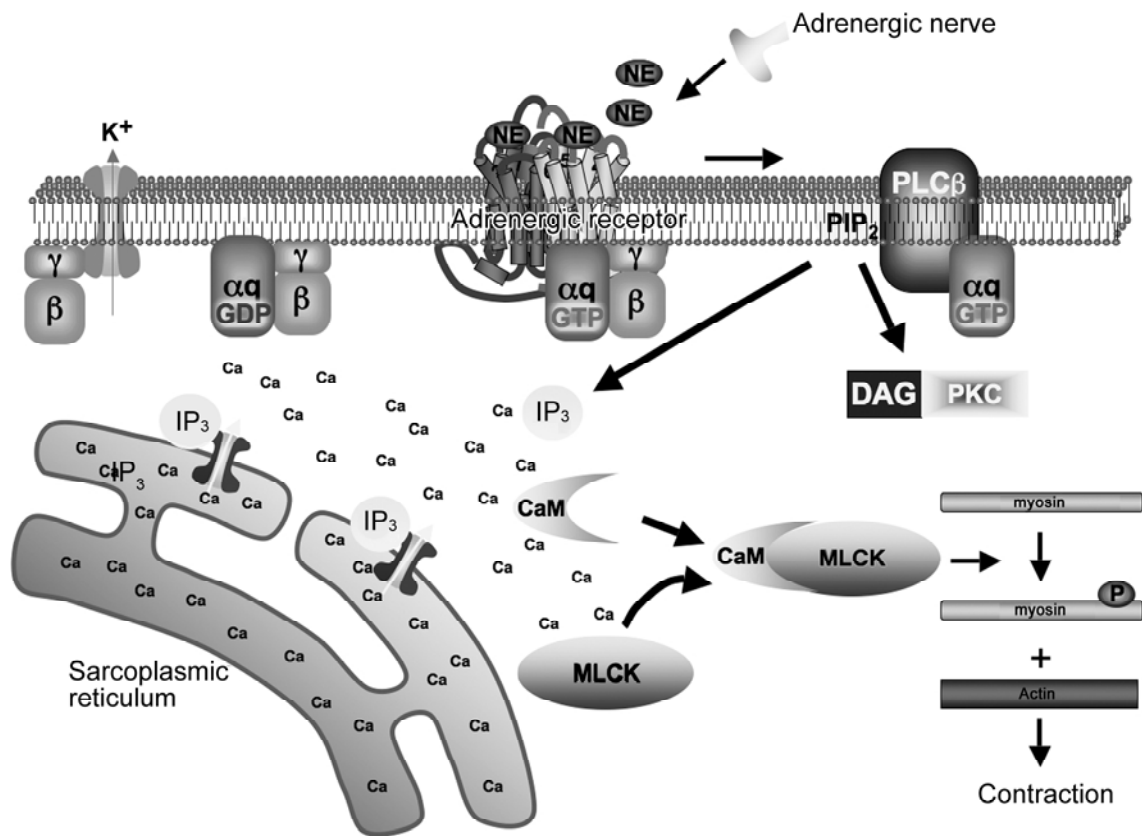


Figure 2. Signaling pathways leading to cavernous smooth muscle (CSM) contraction. Norepinephrin binds to adrenergic receptor, which then activates phospholipase C-β (PLCβ), and which splits phosphatidylinositol (4,5)-bisphosphate (PIP₂) into inositol trisphosphate (IP₃) and diacylglycerol (DAG). Binding of DAG to protein kinase C (PKC) leads to cavernous smooth muscle contraction. IP₃ binds to sarcoplasmic reticulum and triggers the release of calcium (Ca). Calcium (Ca) binds to calmodulin (CaM), which then binds to and activates myosin light chain kinase (MLCK); MLCK phosphorylates MLC, which then binds to and activates actin, resulting in contraction.

vates protein kinase G (PKG) [23], which in turn phosphorylates gap junctions, potassium (K) channels, and Ca channels [24]. Phosphorylation of the K and Ca channels leads to an increase of potassium efflux and reduction of Ca influx, respectively [25]. When the cytoplasmic calcium concentration falls below 500 nmol, Ca dissociates from CaM, which in turn dissociates from the MLCK, thus inactivating it. With its kinase being inactivated and its phosphates being removed by phosphatase, the MLC becomes dephosphorylated. Dephosphorylated MLC inhibits the binding of the myosin head to actin, resulting in the relaxation of CSMC [26]. A more detailed discussion of this Yang pathway can be found in the authors' earlier review article [27].

The initial phase of smooth muscle relaxation results in reduced peripheral resistance of cavernosal arterioles and thereby allows blood to flow into the penis under the

driving force of systemic blood pressure. The increased blood flow causes shear stress that causes endothelial cells to release additional NO [28], which augments the ongoing PKG signaling pathway. As a result, sinusoidal spaces are filled with blood, creating a pressure to compress the venules against the tunica albuginea, limiting venous outflow [29]. This veno-occlusion mechanism, together with the ongoing arterial inflow, leads to a dramatic increase of the intracavernosal pressure and therefore a sustained erection.

After ejaculation or discontinuation of the sexual impulse, NO release from cavernous nerves and endothelial cells ceases or declines, resulting in a drop of cGMP production in CSMC. Meanwhile, the pre-existing cGMP is hydrolyzed to guanosine monophosphate by phosphodiesterase 5 [30], thereby depleting the cGMP store and returning its downstream targets to the deactivated state

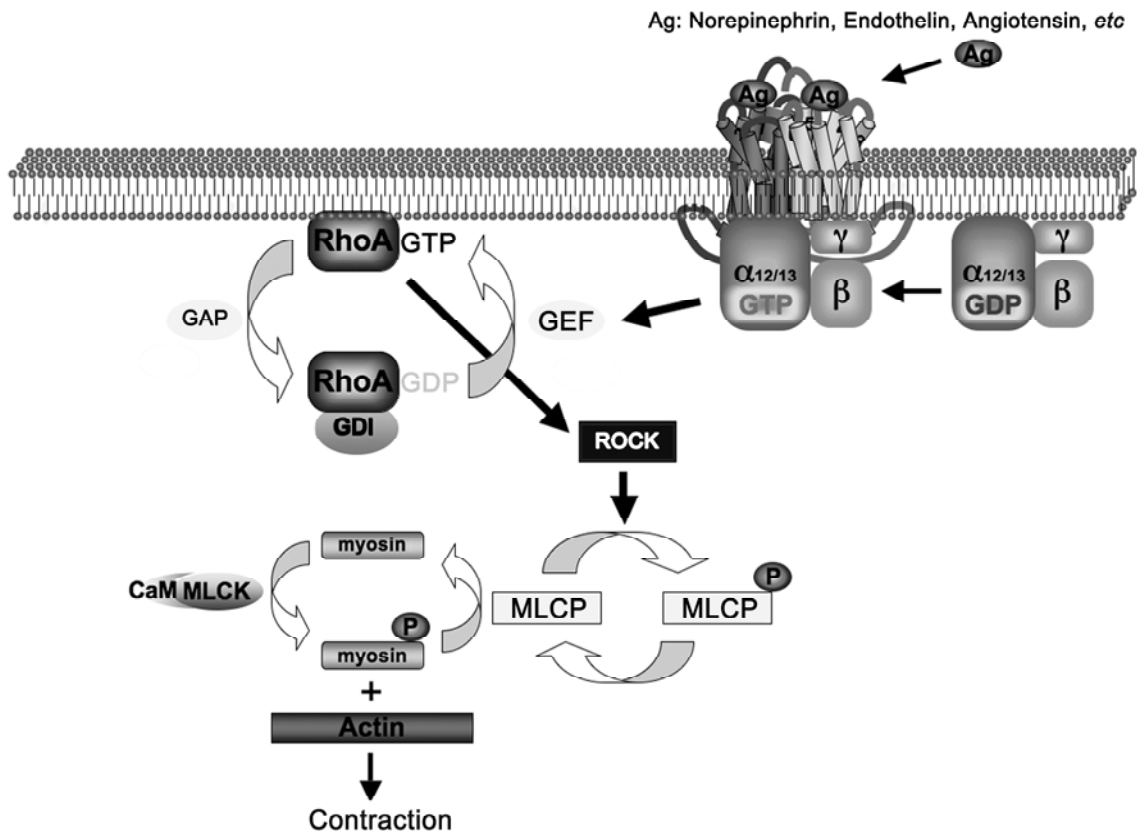


Figure 3. Signaling pathways maintaining cavernous smooth muscle (CSM) contraction. Norepinephrin, endothelin-1 and angiotensin II bind to their respective receptors, leading to the activation of guanine exchange factor (GEF), which converts RhoA-guanosine diphosphate (GDP) to RhoA-guanosine triphosphate (GTP). RhoA-GTP dissociates from GDP dissociation inhibitor (GDI) and migrates to the cytoplasmic membrane, where it binds to and activates Rho kinase (ROCK). ROCK phosphorylates and inactivates myosin light chain phosphatase (MLCP), allowing MLC to stay phosphorylated and consequently actin-contracted. Ag, agonists.

in CSMC. As the Yang forces retreat, the Yin forces advance (see above), and the erection cycle is completed.

The above-described contraction/relaxation processes are responsible for “normal” erectile function, but alternative Yin and Yang mechanisms do exist. For example, intracavernosal prostaglandin E (PGE) injection is one of the most effective treatments for erectile dysfunction (ED) [31]. However, PGE signals through the cyclic adenosine monophosphate (cAMP), not cGMP, pathway. Binding of PGE to its receptor, also belonging to the GPCR family, activates adenylyl cyclase, which converts adenosine triphosphate to cAMP [32]. Binding of cAMP to protein kinase A results in protein kinase A activation, which phosphorylates Ca and K channels, with subsequent events that are similar to the above-described cGMP pathway. Another example is the identification of

natriuretic peptide receptors in CSMC [33, 34]. These receptors are called particulate guanylyl cyclase (pGC) because they are cytoplasmic membrane-bound GC. Soluble guanylyl cyclase is activated by NO (see above), but pGC is activated by natriuretic peptides. Activated pGC converts GTP to cGMP, which activates PKG, and so forth, as described above for the NO–cGMP pathway.

The Yin–Yang philosophy also stipulates that Yin and Yang are interdependent and interact with each other, or “cross-talk”, as molecular biologists would say. Evidence for Yang intercepting the Yin pathway is presented with the discovery that PKG phosphorylates and thereby inhibits RhoA [35], thus suppressing the Yin forces. However, ROCK (a Yin molecule) can inhibit both the expression and activity of endothelial nitric oxide synthase (eNOS; a Yang molecule) [36–38], thus decreas-

ing NO production.

3 Yin and Yang of ED

By far the most important concept in the Yin–Yang philosophy is that, in order for an entity to be wholesome, Yin and Yang must exist in balance in that entity. In fact, traditional Chinese medicine, be it diagnostic or therapeutic, is entirely based on this concept. So, for the penis to be wholesome, Yin and Yang must exist in balance – too much Yin, one gets ED; too much Yang, priapism. ED is known to be associated with aging and several pathological conditions such as diabetes, hypertension, hypogonadism, hypercholesterolemia, and prostatectomy-caused injuries. Aging is associated with decreased neuronal nitric oxide synthase (nNOS) expression in the corpus cavernosum [39]. Diabetes is associated with inactiva-

tion of eNOS [40] and decreased PKG activity [41]. Hypogonadism is perhaps associated with decreased nNOS and eNOS expression [42–44], although contradictory findings also exist [45, 46]. Hypercholesterolemia does affect nNOS or eNOS expression [47] but is associated with a decreased level of phosphorylated (functional) eNOS [48]. Finally, injury to the cavernous nerves results in decreased nNOS expression [49, 50].

The above examples are medical conditions in which lowered levels of Yang molecules are associated with ED, but increased levels of Yin molecules have also been identified in these conditions. For example, MLCP phosphorylation is markedly increased [51], thus in favor of contraction, in the penis of aged rats. RhoA/ROCK activity and MLCP phosphorylation are increased in the penis of diabetic rabbits and rats, respectively [13, 38]. RhoA expression and RhoA/ROCK activity are increased in the

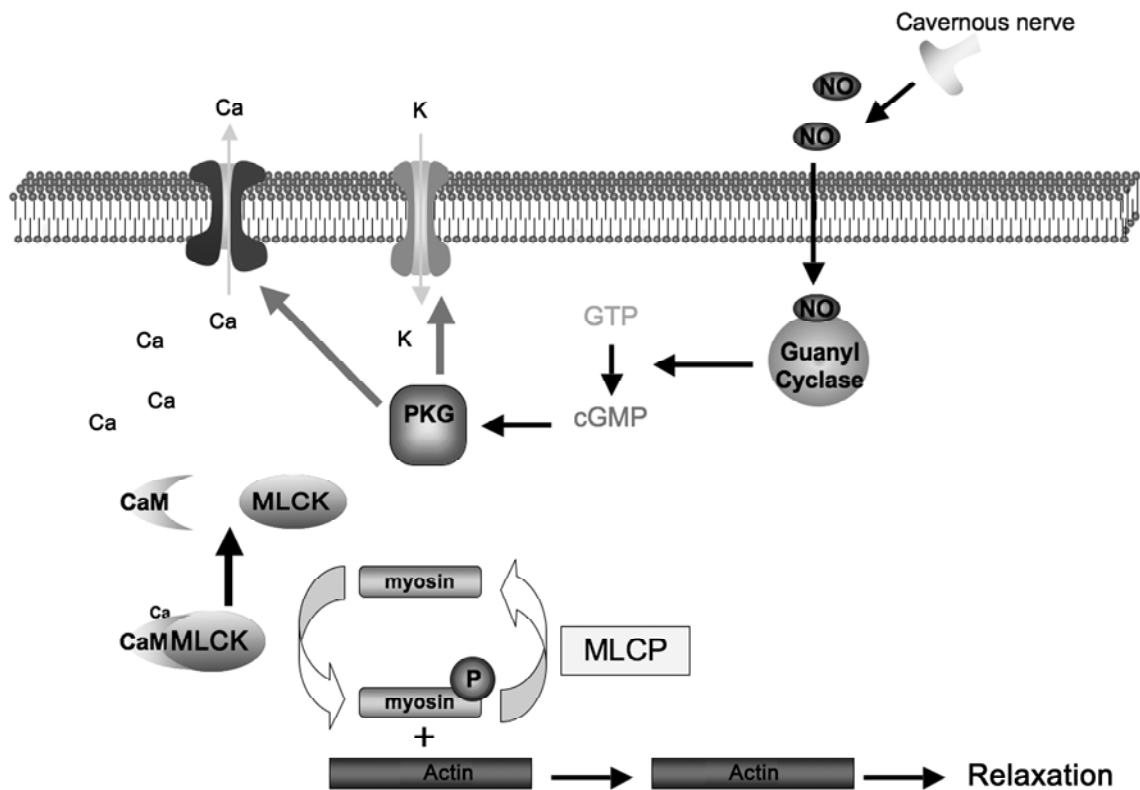


Figure 4. Signaling pathways leading to cavernous smooth muscle (CSM) relaxation. Sexual stimulation triggers the release of nitric oxide (NO) from cavernous nerves in the penis. NO diffuses into CSM cells (CSMC) and activates guanyl cyclase, which then catalyzes the conversion of guanosine triphosphate (GTP) to cyclic guanosine monophosphate (cGMP). cGMP activates protein kinase G (PKG), which in turn phosphorylates potassium (K) and calcium (Ca) channels. Phosphorylation of K and Ca channels leads to an increase of potassium efflux, a reduction of calcium influx, and the dissociation of calcium from calmodulin (CaM), which in turn dissociates from the myosin light chain kinase (MLCK), thus inactivating it. Inactivation of MLCK and removal of phosphates by myosin light chain phosphatase (MLCP) lead to dissociation of myosin from actin and relaxation of actin.

penis of hypertensive [52, 53] and castrated rats [54].

Sickle cell anemia is a well-known risk factor for priapism [55]. The possible underlying mechanism is that sickle cell anemia causes tissue ischemia, which inhibits CSMC contraction [56]. In support of this hypothesis, we have previously reported that CSMC express much lower levels of phosphodiesterase 5 when cultured under low oxygen (hypoxia) conditions [57]. More recently, we discovered that hypoxia also causes lowered expression of RhoA, ROCK, and MYPT1 (the regulatory subunit of MLCP) in CSMC and CSM (Lin *et al.*, unpublished data, 2007). Thus, it appears that insufficient expression of Yin molecules is a possible cause of priapism.

4 Concluding remarks

Despite being thousands of years old, the Yin–Yang philosophy is still applicable to modern medicine in which all sorts of health problems are being investigated for their underlying causes at the molecular level. Although the penis is undoubtedly a Yang organ, it might come as a surprise to some that it actually contains both Yin and Yang molecules, and Yin is actually the dominant force. Thus, it is advisable that we carry out our research by looking not only at the Yang side but also the Yin. Although boosting the Yang has been the preferred route to treat ED, it is possible that reducing the Yin could work just as well. By balancing the Yin–Yang seesaw, one should be able to achieve the Tai Chi state of perpetual happiness.

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