Gene Transfer for the Treatment of Erectile Dysfunction.

7

Albert Einstein College of Medicine

Arnold Melman

1.0 Introduction

1.1 Erectile Dysfu

Erectile Dysfunction (ED) is a disease defined as the inability to achieve or maintain an erection sufficient for satisfactory sexual function. ED is a common and significant problem that afflicts approximately 20 – 30 million men in the United States and 150 million men worldwide.[1-4] Overall, 52% percent of men have some degree of erectile dysfunction and 25% of 75-year old men have complete erectile dysfunction. The most common cause of ED is advanced age. The second most common cause is diminished blood flow to the penis due to cardiovascular disease.

Treatments for ED include: 1) oral sildenaphil (Viagra^{TM}); 2) vacuum erection devices; 3) intraurethral alprostadil; 4) intracavernous injection of prostaglandin E1 (PGE1) or a combination of PGE1, papaverine and phentolamine; and 5) surgical placement of penile prosthetic implants for physiological disease. Despite the availability of these therapies, lack of efficacy, their potential side effects, and loss of spontaneity have limited therapy for ED to about 10% of the overall patient population with ED.

1.2 Role of Ion Channels in Erectile Dysfunction

Why are contraction and relaxation important to an erection and what is the role of the Maxi-K channel in this process? Contraction and relaxation of smooth muscle, is critical to the storage and/or conduit function(s) of hollow organs such as the bladder, gut, blood vessels and penis. K channels play an important role in this process by virtue of their ability to alter the membrane potential and excitability of smooth muscle cells.[5-7] Their primary effect is to modulate Ca^{2+} influx through Ca channels (i.e., L-Type, voltage-dependent). The amount of Ca^{2+} that enters the cell through these channels is a major determinant of the free intracellular calcium levels inside the smooth muscle cell, which in turn, determine the degree of smooth muscle cell contraction. Overall, regulation of the contractile process is organ-specific and modulated by a variety of K channel subtypes.[8-11] Prominent amongst the K channel subtypes found in smooth muscle is the large conductance, calcium sensitive K channel, referred to as the maxi-K channel. Increased maxi-K channel activity is associated with corporal smooth muscle cell relaxation and penile erection. Moreover, alterations in K channel physiology/function are increasingly being recognized as a major contributing factor to the development of the vascular pathologies associated with diabetes,[12,13] as well as urogenital disorders including erectile dysfunction .[5,6,9]

Intracellular concentrations of ions and second messengers (e.g., K^+, Ca^{++}, cAMP, cGMP, etc) are affected by release of neurotransmitters and in turn the changes can be rapidly propagated from cell to cell through specialized intercellular protein pores called gap-junctions.[15-17] The importance of gap junctions to coordinate smooth muscle function cannot be overstated. Specifically, gap junctions are critical not only to the success of all forms of currently available pharmacotherapy, but also to the proposed ion channel gene transfer protocol. This supposition is reflected by the fact that even after severe nerve loss

such as can occur with diabetes or radical prostatectomy there remains sufficient level of intercellular coupling to allow the corporal tissue to function normally following the presentation of an appropriate (i.e., sufficient) membrane or receptor stimulation. For example, oral administration or by intracavernous or intraurethral injection of a drug that stimulates the exchange of ions across the cell membranes can allow the penis to become erect. This approach has proven successful with FDA approved intracavernous (Edex^{TM}, Caverject^{TM}) and intraurethral (Muse^{TM}) injection with prostaglandin E1.

In short, cellular mechanisms that influence cell membrane potential and take advantage of the presence of intercellular communication are of critical importance in regulating the moment-to-moment tone of any organ composed predominantly of smooth muscle whether it is the penis, urinary bladder, blood vessels or gut.

1.3 Maxi-K Channels

At least four K channel subtypes are present in human corporal smooth muscle cells. These channels include: (1) the voltage and calcium-sensitive maxi-K, (2) ATP dependent K channels (K_{ATP}), (3) inwardly rectifying channels (Kir), and (4) voltage-gated K channels (K_V).[7] The primary function of K channels is to modulate Ca^{++} influx through Ca-channels (i.e., L-Type, voltage-dependent). The amount of Ca^{++} that enters the cell through these channels is a major determinant of the free intracellular calcium levels inside the smooth muscle cell, which in turn, determine the degree of smooth muscle cell contraction.

Overall, regulation of the contractile process is organ-specific and modulated by a variety of K channel subtypes. [11,17-19] The maxi-K channel is a prominent (numbering approximately 1000 to 1500/cell[17]) and well-studied K-channel subtype involved in corporal smooth muscle relaxation. Increased maxi-K channel activity is associated with corporal smooth muscle cell relaxation and penile erection. Moreover, alterations in K channel physiology/function are increasingly being recognized as a major contributing factor to the development of the vascular pathologies associated with diabetes [12,13] as well as urogenital disorders [9] including erectile dysfunction. [6, 20]

The maxi-K channel is composed of α and β subunits.[7] The α, or pore forming, unit of the maxi-K channel is a tetramer of homologous units. Each α subunit is composed of 11 hydrophobic domains. Seven of the domains are transmembrane spanning (S0-S6) and the remaining 4 domains are cytoplasmic (S7-S10). The α subunit has intrinsic sensitivity to Ca^{++} via the cytoplasmic tail region.[21] Even in the absence of the β subunit, expression of the α subunit of the maxi-K channel can form a functional unit; however, expression of the β subunit alone does not form a functional channel.[12] It has been proposed that the role of the β subunit is to modulate the α subunit activity or sensitivity to Ca^{++}. [22]

1.4 Rationale for Development of *h*Maxi-K

Ion Channel Innovations has developed a gene transfer product, *h*Maxi-K, and plans to investigate the effect of increased expression of maxi-K channels in the smooth muscle of the penis in patients with ED. *h*Maxi-K consists of the gene for the α pore of the maxi-K channel, *hSlo*, inserted into a plasmid vector, pVAX. Because heightened smooth muscle tone may be a causative factor of erectile dysfunction, increased numbers of maxi-K

channels following gene expression of *h*Maxi-K may effectively correct ED.[23, 24] This approach to treat ED uses the same principal as all drugs developed since 1982, i.e., treatment with medicines causing relaxation of the penile smooth muscle results in rigid erections.

How does Maxi-K channel gene transfer work? The rationale for the utility of K channel gene therapy is related to the important contributions that ion channels make to the contraction and relaxation of smooth muscle cells (i.e., myocytes). Ion channels are membrane proteins that provide a selective permeability barrier to the movement of ions across the cell membrane (influx and efflux of ions, i.e., K^+ and Ca^{2+}.) In short, these membrane proteins provide a selective channel through which ions can flow (K^+ flows through K channels, and Ca^{2+} flows through Ca channels, but not vice versa). The opening and closing of these channels is regulated by numerous cellular processes. However, anything that increases the extent that they are open will increase the amount of ion that can move through the channel over any given period of time. The idea behind maxi-K channel gene transfer is to increase the number of maxi-K channels in the cell membrane, so that when the cells are activated by the normal erectile stimulus (i.e., nitric oxide released from nerves), there will be an increase in the efflux of K^+ from the cell. The rationale for this approach is depicted in Figure. 1.

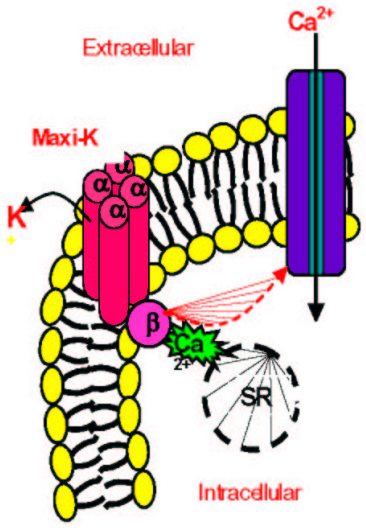

Figure 1. Schematic depiction of the role of the maxi-K channel in modulating transmembrane calcium flux and free intracellular calcium concentration. The free intracellular calcium concentration is an important determinant of smooth muscle cell tone. An increase in the intracellular calcium level is associated with increased smooth muscle contraction, and a decrease in intracellular calcium levels is associated with a decreased smooth muscle tone (relaxation). In smooth muscle the outward movement of K^+ causes a net movement of positive charge out of the cell, making the cell interior more negative with respect to the outside. This has two major effects: First, the increased membrane potential ensures that the calcium channel spends more time closed than open. Second, because the calcium channel is more likely to be closed, there is a decreased net flux of Ca^{++} into the cell and a corresponding reduction in the intracellular calcium levels. The reduced intracellular calcium leads to smooth muscle relaxation. Having more maxi-K channels in the cell membrane should lead to greater smooth muscle cell relaxation.

Transfection and functional activity of the *hSlo* gene has been demonstrated in several *in vitro* experiments. Intracellular uptake of the plasmid, expression of the *hSlo* gene and localization of maxi-K channels to the cell membrane was demonstrated in transfected Xenopus oocytes and human embryonic kidney cells in our preclinical studies (Christ et al., unpublished observations). The expressed channels had all of the expected properties of the native maxi-K channels.[25] Endothelin-1 stimulation induces a transient increase in the intracellulular calcium concentration.[26, 27] Expression of the *hSlo* gene transfected into cultured human corporal smooth muscle cells resulted in significant reduction in that resting and endothelin-stimulated calcium concentration; consistent with the putative mechanism of action of this potent vasoactive peptide in the penile corpora. (unpublished observations).

Again, consistent with the putative mechanism of action, the ability of *hSlo*-plasmid vector to elicit physiologically relevant changes in erectile capacity, has been demonstrated in experiments using the aged rat and streptozotocin-induced diabetic rat models of ED.[23, 24] Thus, animals administered the *hSlo* gene were able to achieve penile rigidity following both electrical stimulation of the cavernous nerves and the medial pre-optic area of the hypothalamus (a region of the brain that initiates erectile activity). Functional activity of *hSlo* gene transfer was also shown in normalization of erectile response in treated aged rats given apomorphine.[28] Apomorphine is known to cause pharmacological stimulation of the dopamine D_2 receptors of the paraventricular nucleus resulting in erection.

Results from these preclinical studies suggest that expression of the α pore for the maxi-K channel following *hSlo* gene transfer should prevent or diminish the effects of aging on the erectile mechanism.[24] A single administration of *hSlo*-plasmid to aging rats with ED has been shown to have bioactivity at least up to six months.[24] This long-term activity of *hSlo* gene expression may represent a distinct advantage over current drug therapy for ED where the effect of treatment is temporary.

3.1 *In vitro* Preclinical Pharmacology Studies

The ability of pcDNA/*hSlo* to transfect cells, express *hSlo*, and localize the maxi-K channel to the cell membrane was demonstrated in *in vitro* experiments using the 293 human embryonic kidney cell (HEK293) and Xenopus oocytes. Xenopus oocytes have few or no known maxi-K channels and HEK293 is a human cell line that also lacks expression of maxi-K.

Following transfection of HEK293 cells, expression of *hSlo* was demonstrated by Western analysis of SDS-PAGE gels with a commercially available chicken anti-maxi-K. Immunocytochemistry staining of transfected HEK293 cells showed that expressed maxi-K channels localized to the cell membrane. (unpublished observations). The functionality of the expressed maxi-K channels was demonstrated in experiments in transfected Xenopus oocytes. Single cell voltage clamp experiments showed K+ efflux from the transfected oocytes [25]

Modulation of calcium entry following expression of maxi-K channel was demonstrated in experiments using human corporal smooth muscle cells transfected with pcDNA/*hSlo*. Endothelin-1 (ET-1) is a potent vasoconstrictor peptide known to increase intracellular calcium levels in cultured human corporal smooth muscle cells and elicits long-lasting contractile responses on isolated human corporal tissue strips. Thus, ET-1 is thought to be an important modulator of flaccidity *in vivo*. [26-28] A significant decrease was observed in both the resting Ca^{++} levels and the peak amplitude of the ET-1 – induced intracellular Ca^{++} transient in *hSlo*-transfected human corporal smooth muscle cells. (unpublished observations). These results suggest that an increase in the number of functional maxi-K channels in the membrane following *hSlo* expression decreases the function of voltage-dependent Ca-channels that, in turn, reduces calcium influx and lowers the intracellular calcium concentration, promoting corporal smooth muscle relaxation.

3.2 *In vivo* Preclinical Pharmacology Studies

The effect of gene transfer of *hSlo* on erectile response *in vivo* was evaluated using two preclinical models of erectile dysfunction: streptozotocin-induced diabetic rats and retired breeder Sprague-Dawley rats.[23, 24] The effect of single intracorporal doses of 10, 30, 100, 300 and 1000 μg of pcDNA/*hSlo* plasmid on the ICP/BP ratio was compared to controls. In these experiments the Intracorporal Pressure/Blood Pressure (ICP/BP) ratio was measured following external electrostimulation of the cavernous nerve.

In order to achieve an erectile response, the arterial and cavernous smooth muscle cells of the penis must be sufficiently relaxed to permit transmission of systemic pressure into the corpus cavernosum. As long as the corporal veno-occlusive mechanism functions properly, blood engorges the corporal spaces under systemic pressure and the penis becomes rigid. The ICP/BP ratio is a physiological measurement that correlates with engorgement of the corpora with blood. Following an extensive series of experiments, a visually observable erectile response was seen in the majority of rats when the ICP/BP ratio was ≥ 0.5 while a visually observable erectile response was seen in all animals regardless of age when the ICP/BP ratio was ≥ 0.6.

In humans, a similar measurement, the Penile to Brachial Index (PBI), has been used to correlate with penile rigidity.[29-34] A PBI ratio <0.6 has been shown to be insufficient for penile rigidity to occur. While PBI and ICP are not directly equivalent, it is noteworthy that their values and physiological significance are qualitatively similar in humans and rats. Thus, the ICP/BP ratio is an objective measure of penile rigidity and can be used to evaluate the effect of gene transfer with *h*Maxi-K on penile rigidity.

3.2.1 Peripheral Neural Stimulation Studies

The effect on the ICP/BP ratio of a single intracorporal injection of pcDNA/*hSlo* followed by external electrostimulation of the cavernous nerve was evaluated in the streptozotocin (STZ)-induced diabetic rat and retired breeder Sprague-Dawley rat models.

In the STZ-induced diabetic rat model, rats confirmed to be diabetic 2 months following STZ-induced diabetes were injected with pcDNA/*hSlo* plasmid. Systemic blood pressure and nerve-stimulated ICP's were measured at various stimulus intensities ranging from 0.5 – 10.0 mA at monthly intervals ranging from 1 to and 4 months following injection of plasmid DNA. Age matched non-diabetic rats, untreated STZ-diabetic rats, and STZ-diabetic rats injected with 1000 μg of the pcDNA plasmid backbone alone served as controls.

Overall, cavernous nerve stimulation in STZ-diabetic rats 2 months following intracavernous injection of *hSlo* resulted in ICP/BP ≥ 0.6 in the gene therapy groups while the STZ-diabetic controls had ICP/BP ratios <0.6. Analysis of the tested doses of pcDNA/*hSlo* on the ICP/BP ratio demonstrated the following: 1) The 10 μg dose is near the threshold for physiological effect; 2) The 30-1000 μg treatment groups consistently had ICP/BP ratios significantly greater than those observed in the untreated groups, and in a large majority of cases these values were greater than 0.6; 3) Response was seen to last at least to 4 months following study drug administration but the magnitude of the response elicited by all gene therapy doses declined with elapsed time post-injection. Although not formally measured in this study, it was noted that the majority of *hSlo*-treated rats had visible erectile responses in contrast to the untreated rats where fewer visible erections were noted.

Retired breeder Sprague-Dawley rats typically display a significant age-related decline in erectile capacity.[24] In this second animal model, the ICP/BP ratio was measured in male Sprague-Dawley rats >500g, and older than 9 months who received a single intracorporal injection of 10, 30, 100, 300 and 1000 μg pcDNA/*hSlo* plasmid or 1000 μg pcDNA plasmid as control. Cavernous nerve stimulation ranged from 0.5-6 mA. Similar to the STZ-induced diabetic rat model, a dose-related effect on ICP/BP ratio was seen in the retired Sprague-Dawley rat model. The 10 μg pcDNA/*hSlo* dose was less effective than the higher doses. With time, the overall effect of all doses decreased and greater levels of neuronal stimulation (i.e., 6 mA current stimulation) were needed to elicit a response. In all cases, more *hSlo*-treated rats had visible erectile responses in contrast to the untreated rats that also had ICP/BP ratios < 0.5-0.6.

In these two distinct animal models of ED, increased expression of maxi-K channels following *hSlo* gene expression resulted in increased ICP/BP ratio. For most doses evaluated, the increase in ICP/BP ratio was to a level that has been shown to correlate

physiologically with penile erection. Importantly, in both models the ICP (erectile) response seen with cavernous nerve stimulation returned to basal (resting) levels immediately upon termination of the external, peripheral neural stimulus. This return to baseline intracorporal pressure suggests that potentially undesirable effects (e.g., priapism) due to expression of *hSlo* in the corporal smooth muscle cells of the penis are unlikely to be seen in the clinic.

3.2.2 Central Neural Stimulation Studies

In order to assay the effect of pcDNA/*hSlo* on the erectile response under conditions that might better mimic natural stimulation for penile erection, two experiments were conducted using either direct central neural stimulation or apomorphine administration with measurement of the ICP/BP ratio in rats administered a single injection of 100 μg pcDNA/*hSlo*. In these experiments we stimulated the medial preoptic area (MPOA; via direct electrical stimulation) and the paraventricular nucleus (PVN; via pharmacological stimulation) of the hypothalamus, which are both known to be important to initiating/integrating erectile capacity in humans and rats.[35] The drug apomorphine has a higher affinity for D_2-like receptors than other dopamine receptor subtypes (i.e., D1-D5) and it is this receptor subtype that is thought to be the main site for the induction of erections in the paraventricular nucleus of the hypothalamus .[36,37] Apomorphine is therefore postulated to increase erectile responses by acting as a conditioner in the PVN, increasing the response to sexual stimuli resulting in enhanced erections

Retired breeder Sprague-Dawley rats were injected intracorporally with 100 μg pcDNA/*hSlo*. Following electrostimulation of the MPOA, expression of *hSlo* in the rat corporal tissue was confirmed by RT-PCR. Rats injected intracorporally with pcDNA/*hSlo* had significantly higher ICP response compared to untreated age-matched control animals following central stimulation. There was no significant difference in the ICP/BP between the *hSlo*-treated and young control animals following MPOA electrostimulation.

3.4 Summary of Preclinical Studies

Ion Channel Innovations has performed numerous pre-clinical studies evaluating the activity and safety of *hSlo* gene transfer at doses up to 1000 μg. From these studies the following conclusions can be made:

- Transfection of cells with pcDNA/*hSlo* is followed by expression of the *hSlo* gene and localization of functional maxi-K channels into the cell membrane.
- Administration of pcDNA/*hSlo* into the corpora of rats followed by electrostimulation of the cavernous nerve or the MPOA of the hypothalamus or pharmacological stimulation of the PVN with apomorphine significantly increased ICP/BP ratio compared to controls.
- Repeat administration of 100 μg plasmid-*hSlo* gene intracorporally did not appear to increase the ICP/BP ratio more than a single 100 μg dose and was not associated with detectable adverse effect on clinical cardiovascular parameters.

- A single administration of 10, 30, 100, 300, or 1000 μg pVAX/*hSlo* (*h*Maxi-K) was well tolerated and was associated with no tissue changes in major organs.
- Sporadic detection of pVAX/*hSlo* (*h*Maxi-K) was detected in a limited number of tissues 24 hours after intracorporal administration but was not detected in the heart or testes 24 hours after intracorporal administration.
- Degradation of pVAX/*hSlo* in human blood occurs rapidly. Within 0.5 hours the plasmid converts to nicked circular plasmid DNA and total degradation occurs within 2 hours.

References

1. Feldman HA, Goldstein I, Hatzichristou DG, Krane RJ, McKinlay JB. Impotence and its medical and psychosocial correlates: results of the Massachusetts Male Aging Study. J Urol 151: 54-61, 1994.

2. Melman A, Gingell JC. The epidemiology and pathophysiology of erectile dysfunction. Urol 161: 5-11, 1999.

3. Feldman, HA, Goldstein, I, Hatzichristou, DG, et al. Impotence and its medical and psychosocial correlates: results of the Massachusetts male aging study. J. Urol 151: 54-61, 1994.

4. Benet, AE, Melman, A. The epidemiology of erectile dysfunction. Urol Clin. North Am 22:699-707, 1995.

5. Christ, GJ. K channels as molecular targets for the treatment of erectile dysfunction. J. Androl. 23: S10-9, 2002.

6. Archer, SL. Potassium channels and erectile dysfunction. Vasc Pharm 38:61-71, 2002.

7. Karicheti, V, and Christ, GJ. Physiological roles for K^+ channels and gap junctions in Urogenital Smooth muscle: Implication for Improved understanding of Urogenital Function, Disease and therapy. Current Drug Targets 2:1-20, 2001.

8. Nelson, MY, Quayle, JM. Physiological roles and properties of K channels in arterial smooth muscle. Am J Physiol 264:C799-822, 1995.

9. Lawson, K., Dunne, MJ. Peripheral channelopathies as target for potassium channel openers. Expert Opinion on Investigational Drugs. 10 : 1345-1259, 2001.

10. Somlyo, AP, Wu, X, Walker, LA, and Somlyo, AV. Pharmacomechanical coupling: the role of calcium, G-proteins kinases and phosphatases. Rev Physiol Biochem. & Pharmacol 134:202-234,1999.

11. Cole, WC, Clement-Chomienne, O. Properties, Regulation, and role of potassium channels of smooth muscle. In A functional view of Smooth muscle, p247-318. Eds Barr, L, and Christ, GJ. JAI Press, Stanford, CT. 2000.

12. Korovkina VP, England SK.: Detection and implications of potassium channel alterations.Vascul Pharmacol. 38:3-12, 2002.

13. Sobey CG. Potassium channel function in vascular disease. Arterioscler Thromb Vasc Biol.21:28-38., 2001.

14. Christ GJ, Brink PR, Melman A, Spray DC. The role of gap junctions and ion channels in the modulation of electrical and chemical signals in human corpus cavernosum smooth muscle. Int J Impot Res 5: 77, 1993.

15. Christ GJ. The penis as a vascular organ. The importance of corporal smooth muscle tone in the control of erection. Urol Clin N Amer: 727-745, 1995.

16 Christ GJ, Moreno AP, Parker ME, Gondre CM, Valcic M, Melman A, Spray DC. Intercellular communication through gap junctions: potential role in pharmacomechanical coupling and syncytial tissue contraction in vascular smooth muscle isolated from the human corpus cavernosum. Life Sci 49:PL195, 1991.

17. Melman, A, Christ, GJ. Integrative erectile biology. The effects of age and disease on gap junctions and ion channels and their potential value to the treatment of erectile dysfunction. Urol Clini N Amer 28:217-231, 2001.

18. Fan, SF, Brink, PR, Melman, A, and Christ, GJ. An analysis of the maxi-K+ (K_{Ca}) channel in cultured human corporal smooth muscle cells. J Urology 153:818-825, 1995.

19. Spektor, M., Rodriquez, Rosenbaum, RS, Wang, HZ, Melman, A, and Christ, GJ. Potassium channels and human corporeal smooth muscle cells tone: further evidence for the physiological relevance of the Max-K channel subtype to the regulation of human corporeal smooth muscle tone *in vitro*. J Urol 167: 2628-2635, 2002.

21. Tanaguchi, J, Furakawa, KI, Shigekawa, M. Maxi-K^+ channels are stimulated by cyclic guanosine monophosphate dependent protein kinase in canine coronary artery smooth muscle cells. Pfluegers. Arch 423:167-172, 1993.

22. Korovkina, KP, England, SK. Molecular diversity of vascular potassium channel isoforms. Clin Exp Pharm Physiol 29:317-323, 2002.

23. Christ, GJ, Rehman, J, Day, N, Salkoff, L, Valcic, M, Melman, A, and Geliebter, J. Intracorporal injection of *hSlo* cDNA in rats produces physiologically relevant alterations in penile function. Am J Physiol 275: H600-608, 1998.

24. Melman, A, Zhao, W, Davies, KP, Bakal, R, Christ, GJ. The successful Long-term treatment of age-related erectile dysfunction with *hSlo* cDNA in rats *in vivo*. J Urol 170:285-290, 2003.

25. Shipston, MJ, Duncan, RR, Clark, AG, Antoni, FA, Tian, L. Molecular components of large conductance calcium-activated potassium (BK) channels in mouse pituitary corticotropes. Mol Endocrinol 13:1728-1737,1999.

26. Kim, DC, Gondre, CM, Christ, GJ. Endothelin-1-induced modulation of contractile responses elicited by an α_1-adrenergic agonist on human corpus cavernosum smooth muscle. Int. J. Impotence Res., 8:17-24, 1996.

27. Zhao, W & Christ, GJ: Endothelin-1 as a putative modulator of erectile dysfunction. II. Calcium mobilization in cultured human corporal smooth muscle cells. J. Urol., 154:1571-1579, 1995.

28. Sato, Y., Day, N., Valcic, M., Melman, A., Christ, G.J. Gene therapy with maxi-K channels restores diminished erectile response in older animals during electrical stimulation of the medial pre-optic area (MPOA). J Urol 165: 220 (A), 2001.

29. Abber, JC, Lue, TF, Orvis, BR, McClure DR, & Williams, RD. Diagnostic tests for impotence: A comparison of papaverine injection with the penile-brachial index and nocturnal penile tumescence monitoring. J Urol. 135: 923-925, 1986.

30. Jevitch, MJ. Importance of Penile Arterial Pulse sound Examination in Impotence. J Urol. 124: 820-824, 1980.

31. Kim, SC, Moon, YT, and Oh, CH. Non-visualization versus normal appearance of cavernous arteries on selective internal pudendal pharmaco-angiograms: comparison with Duplex scanning, cavernosal artery systolic occlusion pressure and penile brachial index. Brit. J Urol. 73: 185-189, 1994.

32. Blaivas, JG, O'Donnell, TF, Gottlieb, P, and Labib, KB. Comprehensive Laboratory Evaluation of Impotent Men. J Urol 124: 201-204, 1980.

33. Schwartz, AN, Lowe, MA, Ireton, R, Berger, RE, Richardson, ML, and Graney, DO. A comparison of penile brachial index and angiography: Evaluation of corpora cavernosa arterial inflow. J. Urol. 143: 510-513, 1990.

34. Goldstein, I, Siroky, MB, Nath, RL, McMillian, TN, Menzolan, JO, Krane, RJ. Vasculogenic impotence: Role of the pelvic steal test. J. Urol. 128: 300-306, 1982.

35. Rampin, O. Mode of action of a new oral treatment for erectile dysfunction: apomorphine SL. BJU Int Suppl 3, 22-24, 2001

36. Ariolas, Hedlund, H. The pharmacology and clinical pharmacokinetics of apomorphine SL. BJU. Int 88 suppl 3, 18-21, 2001.

37. Chen, KK, Chan, JY, Chang, LS. Dopaminergic neurotransmission at the paraventricular nucleus of hypothalamus in central regulation of penile erection in the rt. J Urol. 162: 237-242, 1999.