Journal of Andrology, Vol. 23, No. 5, September/October 2002

K Channels as Molecular Targets for the Treatment of Erectile Dysfunction

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Received for publication May 2, 2002; accepted for publication May 16, 2002.

Normal erectile function is a complex neurovascular process that is dependent on a delicate balance between the effects of endogenous vasoconstrictor and vasorelaxing agents on the arterial and corporal smooth muscle cells of the penis (Christ, 1995, 1998a, 2000a). Ultimately, co-ordinated vascular smooth muscle relaxation and contraction are required for erection and detumescence, respectively. Any disruption of this balance, resulting in either impaired relaxation or heightened contractility of the corporal smooth muscle, can produce erectile dysfunction. Barring traumatic injury, congenital defects, or extensive fibrosis (perhaps fewer than 5% of all patients with erectile dysfunction), any physiologic process, whether of endogenous or exogenous origin, that leads to full relaxation of the corporal smooth muscle cells will produce an erection sufficient for coitus. This fact has served as the underpinning of the majority of pharmacotherapeutic treatments for erectile dysfunction to date and has emphasized that the tone of the corporal smooth muscle cell is the final common arbiter of the erectile process in the vast majority of impotent men. The goal of this report was to outline the rationale and strategy for employing corporal smooth muscle K channels as molecular targets relevant to the improved understanding, diagnosis, and treatment of erectile dysfunction.

Strategies for the Development of Organ/Tissue-Specific Treatments for Erectile Dysfunction

In the post-Viagra era, the quest for orally administered, penile-selective enhancers/restorers of erectile capacity has become the gold standard for the development of improved therapeutic agents for the treatment of erectile dysfunction. However, the relatively serendipitous discovery of the utility of Viagra for the treatment of erectile dysfunction highlights the fact that there is currently no established algorithm/paradigm for identifying relevant molecular targets for the "next generation" of improved treatment of erectile dysfunction. As outlined below, there are 3 obvious approaches to do so:

1. Identify and leverage tissue-specific expression of molecular target(s).

2. Identify and leverage tissue-specific function of molecular target(s).
3. Identify and leverage disease-related changes in 1 and 2.

Certainly, the overall approach here is quite straight-forward. The idea is to begin by utilizing any of the numerous new molecular technologies available to perform analysis of gene expression (ie, gene chip microarrays) and establish whether or not there is evidence for tissue-specific expression of relevant molecular targets. Such an approach can seem daunting in light of the fact that gene chips contain upward of 7–10 000 genes. However, the recommended approach is to start by mining the tissue/cell-specific genomic database for genes that are already known to be important modulators of corporal smooth muscle cell tone (ie, K channel modulators). Such "directed" gene-based discovery methods are quite efficient for identifying suspected targets, as well as for "screening" potentially novel targets. However, since differential expression does not guarantee differential function, target validation is required, and the recommended experimental path is outlined in Figure 1. If a gene relevant to the control of corporal smooth muscle cell tone is only expressed, or perhaps even preferentially expressed, in the corporal myocyte, then developing a small molecule to activate this target is certainly feasible.

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Figure 1. Schematic depiction of a proposed preclinical algorithm for the identification and verification of physiologically relevant molecular targets for the improved diagnosis and treatment of erectile dysfunction. As illustrated, a multidisciplinary approach is required to ensure target validation at multiple experimental levels, including in vivo studies. Erection is one of a handful of physiological systems that is uniquely amenable to such an approach.

If taking advantage of tissue-specific expression is not possible, then leveraging differences in tissue-specific function is another option. More specifically, just because a given target is expressed in more than one tissue does not imply that it functions similarly in those distinct tissues. The goal here was to evaluate the contribution of a given target to contraction and relaxation of corporal smooth muscle. Thus, the ability to conduct pharmacological experiments in vivo and in vitro on human corporal tissue strips and to compare the results obtained with similar experiments conducted in appropriate animals models make this approach feasible. Again, by analyzing information obtained at a variety of experimental levels (see Figure 1), one can verify or refute the relevance of suspected targets. In short, the multiplicity of experimental approaches that can be applied to the study of corporal smooth muscle make erectile physiology/dysfunction an ideal model system for identification and validation of molecular targets.

Finally, perhaps it is intuitively obvious that if neither tissue-specific expression nor function of a suspected target is relevant under normal circumstances, then age- or disease-related changes in cell/tissue/organ function produce a suitable target(s). Nonetheless, target validation using the algorithm outlined in Figure 1 would still be required.

Rationale for the Utility of Ion Channels as Molecular Targets for the Treatment of Erectile Dysfunction

In light of the importance of the corporal smooth muscle cell to the propagation of the species, it is not surprising that regulation of the degree of tone of this specialized vascular smooth muscle

cell is intrinsically complex (Christ et al, 1997, 1999; Christ, 2000c). Numerous intracellular processes are known to govern corporal myocyte tone (see Figure 2). They include calcium mobilization (Christ et al, 1992; Zhao and Christ, 1995) and calcium sensitization (Chitaley et al, 2001; Mills et al, 2001; Rees et al, 2001, 2002). Nonetheless, ion channels, the integral membrane spanning proteins found on the surface of corporal smooth muscle cells, represent an important convergence point for directly or indirectly affecting virtually all forms of cellular signaling. Ion channels provide an obligate mechanism for regulating cellular excitability (see Figures 2, 3, 4). The ion channels most commonly found in corporal smooth muscle—namely potassium, calcium, and chloride channels—are so named according to their selective permeability to K^+, Ca^{2+}, and Cl^-, respectively. This report focuses on the functionally antagonist role that exists between K and Ca channels and the degree of corporal smooth muscle cell tone.

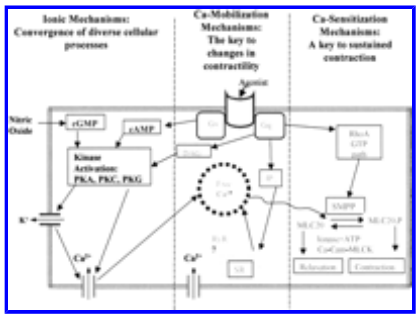

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Figure 2. Schematic of mechanisms known to be important to the regulation of corporal smooth muscle cell tone. The salient mechanisms were arbitrarily subdivided into 3 distinct categories: 1) ionic mechanisms, 2) calcium mobilization mechanisms, and 3) calcium sensitization mechanisms. Certainly, all 3 mechanisms operate simultaneously in the same cell, and all are critical to myocyte contractility. The point to be emphasized here is that by altering ion channel activity, we can predictably modulate smooth muscle tone without ablating contractility altogether. In addition, since virtually all of the second messenger/effector systems ultimately converge on regulation of ion channel activity, pharmacological or genetic modulation of ion channels as a therapy for erectile dysfunction provides a mechanism for regulating smooth muscle cell tone that is independent of any particular receptor/effector deficit. The published literature supporting these conclusions has been previously reviewed (Venkateswarlu and Christ, 2001). SR indicates sarcoplasmic reticulum; RyR, ryanodine receptor; SMPP, smooth muscle myosin phosphatase; MLCK, myosin light chain kinase; PKA/C/G, protein kinase A/C/G, etc; DAG, diacylglycerol; IP3, inositoltrisphosphate; Gs/Gq, heterotrimeric G proteins coupled to adenylate cyclase and phospholipase C, respectively; and Rho A, monomeric G protein.

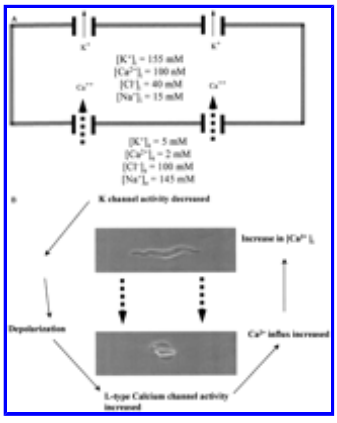

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Figure 3. Relationship between ionic mechanisms and corporal smooth muscle cell contraction. **(Panel A)** illustrates that any stimulus associated with increased contractility will elicit a relative decrease in K channel activity (as illustrated by the small K^+ efflux arrows; compare with Figure 4) and a corresponding increase in Ca channel activity (as indicated by the broader Ca^{2+} influx arrows). The end result of these functionally antagonistic interactions is summarized in **(Panel B)**, which also depicts, via arrows, the corresponding changes in smooth muscle cell shape—in this case, cell shortening.

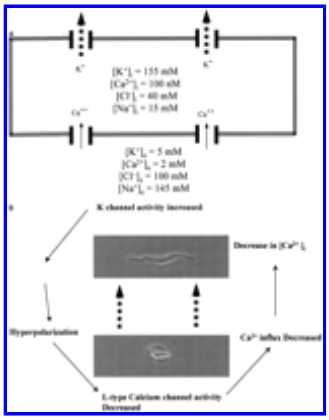

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Figure 4. Relationship between ionic mechanisms and corporal smooth muscle relaxation. **(Panel A)** illustrates that any stimulus associated with increased relaxation (ie, decreased contractility) will elicit a relative increase in K channel activity (as illustrated by the large K^+ efflux arrows; compare with Figure 3) and a corresponding decrease in Ca channel activity (as indicated by the smaller Ca^{2+} influx arrows). The end result of these functionally antagonistic interactions is summarized in **(Panel B)**, which also depicts, via arrows, the corresponding changes in smooth muscle cell shape—in this case, cell lengthening.

The Role of K and Ca Channels in Modulating Corporal Smooth Muscle Cell Tone

The pivotal role of ion channels in modulating membrane potential and corporal smooth muscle cell tone is conferred by the anisotropic distribution of their respective ionic species across the cell membrane. The effects of alterations in K and Ca channel activity on corporal smooth muscle cell tone are therefore a reflection of the standing electrochemical ionic gradients that are maintained by a host of cellular metabolic and biochemical processes (see Figures 3 and 4). Although a detailed review of this subject matter is well beyond the scope of this report, the opening of K channels will, in short, lead to the efflux of K^+ down its electrochemical gradient and out of the corporal smooth muscle cell. Meanwhile, the opening of Ca channels will produce exactly the opposite effect, that is, the influx of Ca^{2+} down its electrochemical gradient. The former moves positive charge out of the corporal smooth muscle cell and leads to hyperpolarization (ie, decreased membrane potential), and thus, reduced cellular excitability, primarily by virtue of the corresponding inhibition of transmembrane calcium flux through L-type voltage-dependent Ca channels (see Figure 4). The latter, or the opening of Ca channels, moves positive charge inside the corporal smooth muscle cell, leading to cellular depolarization (ie, increased membrane potential) and increased cellular excitability (see Figure 3).

The common physiologic link between these 2 functionally antagonistic ion channels, the one that permits them to differentially modulate smooth muscle cell tone, is their respective effects on the free intracellular calcium concentration. In fact, it is now well established that alterations in the free intracellular calcium concentration are an absolute prerequisite to changes in smooth muscle cell tone. More specifically, with respect to the corporal smooth muscle cell, not only are increases in intracellular calcium required for increased contractility, but additionally, sustained contraction of corporal smooth muscle is dependent on continuous transmembrane calcium flux through L-type voltage-dependent calcium channels. The main physiologic implication of the aforementioned interrelationships is that K channels, by virtue of their central physiological location and mechanism of action, provide an ideal molecular target for the treatment of disorders that are characterized by increased smooth muscle cell tone.

Which K Channels Provide the Best Targets?

As observed for other smooth muscle cell types, corporal smooth muscle cells express several distinct K channel subtypes. In addition to the large conductance calcium-sensitive K channel

subtype (maxi-K) and the metabolically regulated K channel subtype (K_{ATP}), previous studies also provide evidence for at least 2 other K channel subtypes (Christ et al, 1993; Malysz et al, 2001). These are an A-type K current, as well as a delayed rectifier current. Although the K_{ATP} and maxi-K channel subtypes apparently account for much of the outward currents observed in cultured and freshly isolated human corporal smooth muscle cells, it would not be surprising if additional K channel subtypes were identified. There is also recent evidence for electrophysiological heterogeneity in the corporal smooth muscle cell population per se (Malysz et al, 2001), and this could have important implications in erectile dysfunction and its therapy. In this report, however, we have focused our investigations on the 2 best characterized and, moreover, arguably the 2 most physiologically relevant K channel subtypes to the regulation of corporal smooth muscle cell tone, namely the K_{ATP} and the maxi-K channel subtypes.

The Presence of Corporal Smooth Muscle Cell Networks Ensures the Tolerance/Efficiency of Heterogeneous Cellular Responses

A series of publications has documented the presence and physiological relevance of gap junctions to the coordination of contraction and relaxation responses among the corporal smooth muscle cells connexin43 (Christ et al, 1997, 1999; Brink et al, 2000; Christ, 2000a,b). Although there are 16 known mammalian gap junction proteins (ie, connexins), the dominant one in human corporal smooth muscle is undoubtedly connexin43. The presence of these aqueous intercellular channels provides partial cytoplasmic continuity between coupled smooth muscle cells and ensures the intercellular transit of most of the known second-messenger molecules/ions that regulate corporal smooth muscle cell tone (Figure 5). Thus, not all smooth muscle cells in the corpus cavernosum need to be directly activated in order to elicit a rapid and syncytial relaxation or contractile response. That is, the smooth muscle cells of the corpus cavernosum function as a syncytial smooth muscle cell network. A correlate of these facts is that not all corporal smooth muscle cells may, or even need to, express the same cellular phenotype. Of direct relevance to this report is that, even if a molecular target is heterogeneously expressed in corporal myocytes, it can still provide an attractive therapeutic target if a sufficient number of cells express that phenotype (eg, this provides an important "safety factor" for the potential success of gene therapy; see below). Theoretical calculations of the number of activated/responsive cells required in order to produce syncytial tissue responses have been previously described (Ramanan et al, 1998).

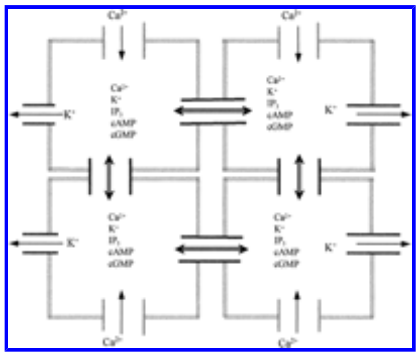

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Figure 5. Schematic diagram that emphasizes the importance of gap junctions to penile erection. As illustrated, intercellular communication provides the anatomic substrate for the establishment of corporal smooth muscle cell networks. These cellular networks are critical to rapid and syncytial contraction and relaxation responses required for detumescence and erection, respectively. In addition, the presence of gap junctions ensures that phenotypic cellular heterogeneity (ie, expression of distinct receptor/effector mechanism on corporal smooth muscle cells) can be tolerated. Thus, the presence of gap junctions guarantees that not all corporal smooth muscle cells need to be directly activated/affected by any given stimulus or therapy; this has important implications to gene therapy of erectile dysfunction (see text).

The Rationale for Pursuing Orally Administered, Organ/Tissue-Selective K Channel Modulators

As reviewed elsewhere, the therapeutic potential of K_{ATP} channel activators to treat vascular disease (ie, increased peripheral resistance) has been established for more than 2 decades (Lawson, 1996). The relatively limited clinical efficacy profile of the K_{ATP} channel modulators is most certainly related to the ubiquitous distribution of the K_{ATP} channel in smooth, skeletal, and cardiac muscle (Lawson, 1996), as well as in numerous other tissues/cell types.

There is some, albeit limited, clinical experience with K channel modulators for the treatment of erectile dysfunction. For example, an orally available maxi-K channel opener was being evaluated in phase 2a study (BMS-223131; Bristol-Myers Squibb, New York, NY), but the outcome of those studies remains uncertain (Pryor and Redmon, 2000). In addition, the possibility of surmounting untoward systemic side effects by utilizing intracavernous injection of a K_{ATP} channel modulator for the treatment of erectile dysfunction (PNU83757; Upjohn, Peapack, NJ) has also been attempted but has yielded somewhat disappointing clinical results as well (Pryor and Redmon, 2000).

Although the initial clinical results are not encouraging, there is reason to believe that a renaissance in the therapeutic utility of K channel activators may soon occur. Such an approach would obviously have to leverage tissue-specific or disease-related changes in corporal smooth muscle K channels. A brief summary of the salient molecular features of the K_{ATP} and maxi-K channel subtypes is therefore given below.

Molecular Composition of the K_{ATP} and Maxi-K Channel Subtypes

Both the K_{ATP} and the maxi-K channels are formed from heteromultimer alpha (α, pore forming) and beta (ß, regulatory) subunits (Figure 6). The K_{ATP} channel has 2 known Kir (Kir; inward rectifier) isoforms (Kir6.1 and Kir6.2) and 4 known regulatory subunits, SUR1, SUR2A, SUR2B, and SUR2C (sulfonylurea receptors [SURs]; Aguilar-Bryan et al, 1998; Babenko et al, 1998; Inagaki and Seino, 1998; Seino, 1999). For example, there are the cardiac-specific (SUR2A/Kir6.2), the pancreatic-specific (SUR1/Kir6.2), and the smooth muscle-specific (SUR2B/Kir6.2 and SUR2B/Kir6.1) complexes that could be targeted by novel, selective K_{ATP} channel openers (Lawson, 2000; Lawson and Dunne, 2001). With respect to the maxi-K channel, only a single isoform of the α subunit has been identified, and only a single ß subunit is present in smooth muscle. But there is evidence of significant physiological distinctions among splice variants in the α subunit; furthermore, there is evidence that the α subunit splice variants exhibit both tissue-specific (Knaus et al, 1995; Wallner et al, 1995; Jones et al, 1998, 1999; Toro et al, 1998; Giangiacomo et al, 2000) and disease-related (Xie and McCobb, 1998) changes.

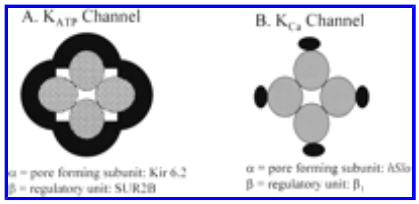

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Figure 6. Schematic depiction of the subunit composition of the K_{ATP} and maxi-K channels (K_{Ca}) in corporal smooth muscle. **(Panel A)**: As illustrated, the α, or pore-forming component of the K_{ATP} channel, is a tetramer of homomeric subunits (stippled; Kir; inward rectifier). The α subunits are associated in a 1:1 stoichiometry with homomeric ß subunits (sulfonylurea receptors, SUR; dark semicircles). **(Panel B)**: As illustrated, the α, or pore-forming component of the maxi-K channel, is a tetramer of homomeric subunits (gray). The α subunits are associated in a 1:1 stoichiometry with homomeric ß subunits (dark ovals).

Disease-related alterations in ion channel function per se have been referred to as "ion channelopathies"—in this case, K channelopathies (Ashcroft, 2000; Lawson and Dunne, 2001). As used herein, the term "K channelopathy" refers to alterations in K channel expression, activity, regulation, or function that provide the mechanistic basis for pathological cellular function—in this case, altered smooth muscle cell tone. The observed tissue-specific expression patterns in the heteromultimer composition of the K_{ATP} or maxi-K channels, as discussed above, are consistent with the possibility that chemical entities may be identified that can leverage these tissue-specific differences in K channel expression or function. Furthermore, if there are disease-related K channelopathies (ie, diabetes), then such an observation may provide a rationale to develop a mechanism-based, patient-specific therapeutic strategy for the treatment of erectile dysfunction. Recent data obtained from human corporal tissue strips have documented the importance of the maxi-K (Spektor et al, in press) and K_{ATP} channels (Venkateswarlu et al, in press) in the contraction and relaxation of human corporal smooth muscle and, furthermore, have suggested that K channelopathy may have an impact on the physiology of human diabetic corporal smooth muscle (Venkateswarlu et al, in press).

So, Can Orally Administered K Channel Activators Provide a Potential Therapy for Erectile Dysfunction?

The activity of the maxi-K and K_{ATP} channel subtypes in corporal smooth muscle is quite low at physiological membrane potentials in the absence of endogenous neurally mediated relaxation (ie, at 40–50 mV, the open probabilities are estimated to be less than 1%; Lee et al, 1999a,b). Moreover, recent studies have documented that the open probabilities of both K channel subtypes are dramatically increased in activity by the addition of prostaglandin E1 (PGE_1) (open probability approaches 1 with 33 μM PGE_1; Lee et al, 1999b) and pinacidil (10 μM), respectively. The apparent quiescence of these channels in this cell type during flaccidity, and yet the ability of physiologically relevant stimuli to increase their activity so dramatically (Lee et al, 1999a,b; Wang et al, 2000), make them attractive molecular targets.

The real question, then, is whether or not it is possible to administer K channel activators systemically at concentrations that would have a greater impact on corporal smooth muscle K channels than on, for example, the vascular K channels in resistance vessels. Stated more succinctly, the real challenge is to identify a therapeutic window that simultaneously satisfies 2 conditions. First, the K channel modulator concentration would have to be low enough so as not to adversely affect the vascular K channels in resistance vessels (ie, no change in blood pressure). Second, the concentration of the K channel modulator achieved in the corporal vascular sinuses would have to be sufficient to ensure that when the K channels are activated by neural signals, an enhanced hyperpolarization and a corporal smooth muscle cell relaxation occur. Preliminary data in our rat model in vivo, using intravenous administration of pinacidil (a selective K_{ATP} channel activator) and NS1619 (a putative selective maxi-K channel activator), are consistent with such a possibility (data not shown). Briefly, intravenous administration of both compounds (0.1–1.0 mg/kg) produced a physiologically relevant increase in the intracavernous pressure response (ICP) to submaximal current stimulation (0.2–0.5 mA) in anesthetized rats. In both cases, prior to administration of the compounds, the nerve-stimulated ICP was insufficient to elicit an erection (ICP = 20–50 cmH_2O), whereas afterward, visible erectile responses were observed in the majority of animals (ICP = 60–100 cmH_2O). Although further experiments are required, these preliminary observations do provide "proof of concept" that such an approach is feasible. As more information is gleaned about the effects of age and disease on the molecular and biophysical properties of these K channel subtypes

in physiologically distinct smooth muscles, it may be possible that one can indeed develop orally activated, tissue-selective K channel activators.

Gene Therapy for Erectile Dysfunction

A recent publication documented that expression of *hSlo* (the α, or pore-forming subunit of the human maxi-K channel; see Figure 6) in a fraction of the specialized vascular smooth muscle cells of the rat corpus cavernosum (ie, corporal myocytes) restored the age-related decline in the nerve-stimulated ICP typically observed in older rats (Christ et al, 1998). Similar observations have been made following 8–12 weeks of experimental diabetes using the streptozotocin-diabetic F-344 rats (Christ, 1998b, 2000b; Melman and Christ, 2001). In both cases, the mechanism of action was hypothesized to be related to the enhanced hyperpolarizing ability of the corporal smooth muscle cell network provided by *hSlo* in response to cellular activation by released neurotransmitters (eg, nitric oxide; see Figures 2 through 4). The fact that relatively low-level *hSlo* transfection rates produced dramatic changes in erectile capacity (ie, intracavernous pressure) was presumed related to the presence and physiological relevance of the intercellular pathway provided by the connexin43-derived gap junction channels (see Figure 5). Thus, not every corporal smooth muscle cell needed to be genetically modified to achieve global affects on tissue function, permitting a certain degree of inefficiency in gene transfer. This inefficiency in gene transfer will likely prove valuable to the safety profile of this novel form of therapy; the smaller the degree of genetic modification required to produce a physiologically relevant effect nominally, the lower the likelihood there will be any untoward effects of the therapy.

Our preliminary observations indicate that the physiologically relevant effects of a single intracavernous injection of naked DNA (ie, *hSlo*/pVAX or pcDNA3) can last for up to 4 months in the diabetic rat (ie, longer time points were not examined) and up to 6 months in the aged rat model. The implication is that a patient could be effectively treated for erectile dysfunction by 1–2 visits to a urologist per year. Certainly, such a therapy would provide clear advantages over other forms of currently available therapy and perhaps over other forms of gene therapy as well (Schenk et al, 2001). Moreover, many impotent men may require no other form of therapy. The increased sensitivity of their corporal smooth muscle cells conferred by expression of the gene product now makes it responsive to the extant neural pathways, whereas prior to gene therapy, the endogenous neural stimulus was insufficient. Even if gene therapy itself were insufficient to restore erectile capacity, the patient could then be placed on other available forms of therapy (ie, Viagra), presumably at lower concentrations, thus minimizing the side effect profile. Such a rationale could well establish a safe and effective gene therapy approach as a first-line treatment for erectile dysfunction and perhaps even as a prophylactic treatment to prevent the onset of impotence.

Summary and Conclusions

K channels appear to provide an ideal molecular target for regulating corporal smooth muscle cell tone and therefore erectile capacity. They do so by virtue of the central role they play in integrating cellular signals and furthermore, because alterations in their activity are commensurate with the modulation, but not ablation, of smooth muscle cell tone. Since both tissue-specific expression and tissue-specific function are characteristics of the multisubunit complexes that comprise the maxi-K and K_{ATP} channel subtypes, significant potential exists for developing novel chemicals that can selectively activate these channels in target tissues. Moreover, the quiescence of these channels during flaccidity and the robust increase in their activity levels during endogenous stimulation bode well for their potential as erectogenic agents. Finally, the ability of intercellular communication through gap junctions to efficiently spread K channel-mediated hyperpolarizing signals throughout the corporal smooth muscle cell network implies that low-

efficiency gene transfer techniques will provide a unique circumstance in which high-efficacy treatments can be locally delivered to the penis, thus further minimizing the potential for systemic side effects.

Appendix

Question 1— About 4–5 years ago, your group reported on gene therapy using maxi-K channels for the treatment of erectile dysfunction. Where are we now in regard to National Institutes of Health (NIH) support and clinical trials?

Answer— There are several other groups working on gene therapy. We have now submitted this protocol to the advisory committee of the NIH and to our institutional review board and hope to recruit human patients sometime later this year.

Question 2— How confident are you that these transfection methods will produce physiologically regulated responses? In other words, could you overdo the response by changing the amount of your channels?

Answer— What I did not show you is that there is a dose-response relationship. If you drop the concentration of transfected gene down to 10 μg, the smooth muscle effect does not last very long. In regard to basal intracavernosal pressure responses, none of the animals we have injected so far had any pathologic effects up to 6 months. We looked at the distribution of the gene in different tissues after intracavernous injections and observed it in the corpus cavernosa and several other places, but within a week, it was gone everywhere else and was expressed at a very low level in the corpus cavernosa. We believe this is a kind of reverse gene therapy. Most researchers working on gene therapy desire the most efficient, most potent, and longest-acting integrative gene they can procure. What we are trying to do is exactly the opposite. We are attempting to achieve the lowest level of expression, counting on the fact that the cells are interconnected by gap junctions and that a small push in the right direction will produce a physiological response during stimulation without any undue adverse effects.

Question 3— Have you seen any priapism in your rat model?

Answer— No.

Acknowledgments

This work was supported in part by NIH USPHS grants DK46379 and DK55076.

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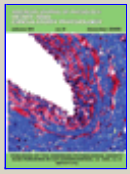

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G. J. Christ, N. Day, C. Santizo, Y. Sato, W. Zhao, T. Sclafani, R. Bakal, M. Salman, K. Davies, and A. Melman
Intracorporal injection of hSlo cDNA restores erectile capacity in STZ-diabetic F-344 rats in vivo
Am J Physiol Heart Circ Physiol, October 1, 2004; 287(4): H1544 - H1553.
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