British Journal of Pharmacology (2006) 147, S56–S61
www.nature.com/bjp

In vitro models: research in physiology and pharmacology of the lower urinary tract

*,[1]Robert B. Moreland

[1]Neuroscience Research, Global Pharmaceutical Research and Discovery, Department R4PM, GPRD, Bldg AP9A Rm 219 Abbott Laboratories, 100 Abbott Park Road, Abbott Park, IL 60064-6123, U.S.A.

The physiology and pharmacology of the lower urinary tract has advanced based, in part, due to the *in vitro* assays that have facilitated this exploration. Such assays have led to the development of novel and selective molecules that have been used to characterize different receptor and enzyme systems in the larger context of *in vivo* pharmacology. These assays can be classified by sites of action of drugs into the following categories: receptors, effector enzymes and enzymes that terminate the responses. In this review, representative assays are presented based on our experience in male erectile dysfunction. *British Journal of Pharmacology* (2006) **147,** S56–S61. doi:10.1038/sj.bjp.0706505

Keywords: GPCR; cAMP; cGMP; ion channel; erectile dysfunction; lower urinary tract

Abbreviations: AA, arachidonic acid; AC, adenylate cyclase; eNOS, endothelial NOS; ELISA, enzyme-linked immunoassay; FLIPR, fluorescent imaging plate reader; sGC, soluble guanylate cyclase; GPCR, G-protein coupled receptor L-Arg, L-arginine; LUT, lower urinary tract; IP3, inositol trisphosphate; MED, male erectile dysfunction; NO, nitric oxide; PDE5, phosphodiesterase 5; PLC, phospholipase C; RIA, radioimmunoassay

Introduction

The lower urinary tract (LUT) functions to store and void urine and plays an important role in male sexual function. Over the last 25 years, the development of small molecules that target specific receptors and enzyme systems has allowed rapid progress in developing an understanding of the physiology and pharmacology of the LUT (see Andersson, 2001; deGroat and Yoshimura, 2001; Moreland *et al*., 2001; 2004a). *In vitro* assays that define both the efficacy and potency at a defined target, as well as the selectivity of that molecule, are key in the development of these small molecules. It is important to note that conclusions derived from complex systems correlate directly to the selectivity of the agents used in the investigation. Over the last decade, highly selective agents that target specific receptors and enzymes have been discovered. It is expected that these agents will help revolutionize the types of questions addressed in LUT physiology and pharmacology. While the various organ systems of the LUT share commonalities, this review will focus on our experience with male erectile dysfunction (MED) as an example of the use of *in vitro* assays in basic science as well as drug discovery. As such this is not meant to be an exhaustive review of *in vitro* assays, but rather illustrative of the principles involved from the experiences in our own laboratory.

Pharmacologically one designates sites of action of drugs into the following categories: (1) receptors (G-protein coupled or not), (2) effector enzymes (e.g. adenylate and guanylate cyclases, phospholipases, kinases, synthases) and (3) enzymes that terminate the responses (e.g. phosphodiesterases, phosphatases). One of the challenges in utilizing isolated *in vitro* assays is the recognition that these receptors and enzymes are integrated into complex pathways (see Eyster, 1998).

*Author for correspondence; E-mail: robert.moreland@abbott.com

The first step in deciding which *in vitro* assays to use is to define the system. A physiologic or pharmacologic observation spurs questions to further investigate the broader role of a receptor or enzyme system in a specific organ system. *In vitro* assays can be broadly classified into binding of ligands or functional assays. If a receptor is involved, one should be able to demonstrate expression, binding of specific ligand or substrates and functional activity. A common misconception is that greater mRNA expression does not imply greater importance. In contrast, it may be that the regulation of the receptor or enzyme by post-translational modification leads to enhanced activity and importance. Recently, functional proteomics has been proposed as a means to examine functional expression in cells and tissues (see Jessani & Cravatt, 2004). While the impact of this technology has yet to be realized, a high throughput platform has been developed (see Jessani *et al.,* 2005). A common theme in characterization of adrenoceptors, muscarinic and prostanoid receptors in corpus cavernosum in our laboratory was demonstration of expression of various receptor subtypes in combination with determining functional responses with known nonselective and selective agonists and antagonists (see below).

Within the assay system, the investigator must exercise care to assure the system chosen reflects, as closely as possible, the *in vivo* situation. Over the course of a quarter century of research, it has been found that the density of G-protein coupled receptors (GPCRs) can affect downstream signaling pathways. Consequently, depending on the system employed, a small molecule may behave as a partial or full agonist, antagonist or inverse agonist (see Kenakin, 2003). Often the choices are limited to transfect the receptor or channel of interest into HEK-293 or CHO-K1 cells. If cellular context (e.g. smooth muscle *versus* neuron) is important, critical information may be missed by assaying the target of interest

into an inappropriate cell type. It is our preference that primary cultures of the tissue of choice be used whenever possible as a secondary assay to confirm results obtained in transfected cells. This is not always possible, however, particularly if a receptor or enzymatic activity is poorly expressed in the tissue of interest.

As penile erection involves relaxation of corporal vascular and smooth muscle during sexual stimulation, the application of *in vitro* models of isolated corpus cavernosal tissue has significantly enhanced our understanding of the biochemical events at the cellular level (see Andersson, 2001; Moreland *et al.,* 2001). From a scientific perspective, these models allow dissection of the various neurotransmitters and vasoactive factors involved in this process and the signal transduction pathways therein (Figure 1). Aside from isolated enzyme preparations, there are three major *in vitro* models currently in use: transfected cells, cell cultures derived from the corpus cavernosum and muscle strips in organ baths (see below). Similarly, in the LUT, cultures of urethral, urinary bladder and ureter cells have been reported, but pharmacology remains largely uncharacterized (e.g. Baskin *et al.,* 1993; Kropp *et al.,* 1999; Corvin *et al.,* 2001). Organ bath studies of ureter, urethra and more common urinary bladder have been reported and the reader is referred to representative examples (Levin *et al.,* 1995; Pontari *et al.,* 2004; Su *et al.,* 2004; Wheeler *et al.,* 2005). Studies of central nervous system components involving brain slices have yet to be used with great utility in this area of research.

During the last decade, primary cultures of corpus cavernosum cells from human or animals have provided useful insights into factors that can modulate the intracellular events. These studies have included cultures of corpus cavernosum endothelial as well as smooth muscle cells. Such experiments have been helpful in defining which receptors are localized in each cell type and in understanding of the specific signal transduction mechanisms. As the degree of smooth muscle tone is mainly controlled by the intracellular Ca^{2+} concentration (Horowitz *et al.,* 1996), any event that alters Ca^{2+} entry to the cell or release of Ca^{2+} from intracellular storage will ultimately have a significant impact on corporal smooth muscle tone (see Andersson & Wagner, 1995). Two primary intracellular messenger molecules (cAMP and cGMP) modulate the continuous transmembrane Ca^{2+} flux through voltage-dependent calcium channels that are critical to the sustained contraction of corporal smooth muscle. Studies in tissue strips and primary cell cultures have provided the available information on intracellular events described in Figure 1.

Receptor assays

Many small molecules are targeted against specific cell surface or nuclear receptors. Pharmacologically, a receptor can be defined as 'a cellular macromolecule, or an assembly of cellular macromolecules, that is concerned directly and specifically in chemical signaling between and within cells' (Neubig *et al.,* 2003). These can include GPCRs, ion channels (discussed below) and nuclear-targeted receptors such as the estrogen, androgen and PPRγ receptors. The two major approaches are utilized to study receptors: binding assays (usually radioligand) and functional assays.

A radioligand binding assay can be used provided sufficient numbers of receptors are expressed and a radioligand is available. The current use of terminology and analysis has recently been reviewed (see Neubig *et al.,* 2003). In the choice of radioligand, many assays use antagonists (such as rauwolscine for α_2-adrenoceptors and spiperone for D_2-like receptors)

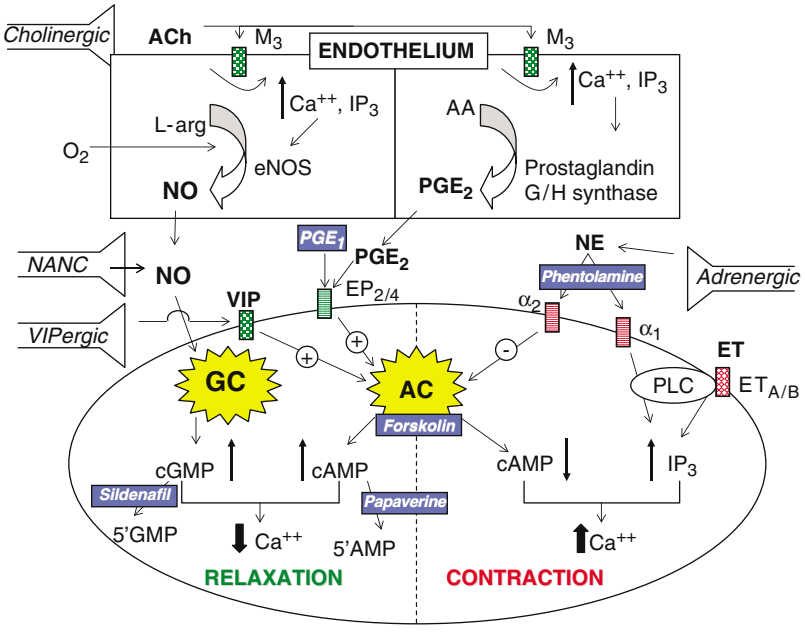

Figure 1 Peripheral mechanisms involved in flaccidity and erection. A conceptual framework depicting mechanisms involved in regulating corpus cavernosum smooth muscle tone as well as the mechanisms of action of peripheral pharmaceutical treatments for MED. AC, adenylate cyclase; AA, arachidonic acid; eNOS, endothelial NOS; GC, guanylate cyclase; L-Arg, L-arginine; IP3, inositol trisphosphate; PLC, phospholipase C. Taken from Moreland *et al.* (2001) (used by permission).

(Traish *et al.,* 1997b; Gupta *et al.,* 1998; Moreland *et al.,* 2004c). In other cases, the investigator may only have available radioactive agonists such as 8-hydroxy-DPAT for 5-HT receptors (e.g. 5-HT_{1A} receptors) or A-369508 for dopamine D_4 receptors (De Vry *et al*., 1998; Moreland *et al.,* 2004c). As in the case of 8-hydroxy-DPAT, rauwolscine and spiperone, the investigator must take care to consider cross reactivity of these ligands with other receptors. Newer, more selective radioligands may obviate this if they are available. The caveats of the employment of an agonist or antagonist radioligand must be taken into account (see Neubig *et al.,* 2003; Kenakin, 2004). Agonist radioligand binding can require an active GPCR (with G protein bound). Depending on the receptor in question, the affinity can differ up several fold to orders of magnitude between the agonist and antagonist used in the assay (Moreland *et al.,* 2004b, c). While antagonist binding is inherently more stable and reproducible, it is important to be aware that differences in affinity for the receptor can exist depending on the ligand used and may affect the overall conclusion. Typically, binding assays involve a timed incubation with the radioligand and membranes expressing the receptor of interest at optimized temperature and conditions. Binding is allowed to proceed until it reaches equilibrium. Termination of the assay can be carried out in a number of ways that separate free from bound and the radioactivity remaining is counted. The end readout is an affinity of the ligand for receptor and utilization of different types of analysis (e.g. Hill, Scatchard, Schild). Using these analyses, one can derive information characterizing ligand affinity as well as the nature of the receptor (high and low affinity, spare receptors, receptor number) (see Neubig *et al.,* 2003; Kenakin, 2004). In basic research, binding assays can provide affinity of sites as well as the total number of binding sites per tissue or cell. In small molecule discovery, binding assays are critical to determine affinity of test small molecules for the receptor in question as well as selectivity at other sites (e.g. see characterization of dopamine D_4 selective agonist radioligand, A-369508; Moreland *et al.,* 2004c).

Functional GPCR assays usually utilize a secondary readout such as GTP-γ-S binding, levels cAMP or cGMP, or calcium influx. Functional assays are performed for shorter periods of time than equilibrium binding assays. GTP-γ-S binding reflects activation of the receptor bound G protein and one of the first steps in signal transduction (e.g. see Newman-Tancredi *et al.,* 2002; Moreland *et al.,* 2004c). Cyclic nucleotides (cAMP or cGMP) assays are usually determined by enzyme-linked immunoassay (ELISA) or radioimmunoassay (RIA). Calcium flux can be measured by use of fluorescent calcium indicators such as Fluo4 and a fluorometer or fluorescent imaging plate reader (FLIPR). Examples in corpus cavernosum smooth muscle cells include $G\alpha_s$ coupled receptors (increase cAMP synthesis) such as the vasoactive intestinal peptide (VIP) receptor, the DP prostaglandin D receptor and EP2 and EP4 prostaglandin E receptors (Moreland *et al.,* 2001; 2002; 2003), $G\alpha_{i/o}$ coupled receptors (decrease forskolin cAMP synthesis), such as α_2-adrenoceptor (Traish *et al.,* 1997b; Gupta *et al.,* 1998) and $G\alpha_{q11}$ coupled receptors (increased calcium flux of phospholipase activity) such as α_1-adrenoceptors and endothelin ET_A and ET_B receptors (Figure 1). In some cases, undetectable amounts of receptors on the cell surface can yield results in functional assay results as has been seen with α_2 adrenoceptors on primary cultures of rabbit corpus smooth muscle cells (Gupta *et al*., 1998). Forskolin induction of cAMP can lead to a problematic readout, even in primary cultures, as some receptors such as the M_2 and M_4 acetylcholine receptors that are $G\alpha_i$ coupled signal *via* $G\alpha_s$ increasing cAMP synthesis (Traish *et al.,* 1995b; 1997a).

Recently, dopamine D_4 receptor agonists have been characterized that induce penile erection by a supraspinal mechanism (Brioni *et al*., 2004). Since this receptor is sparsely expressed in the central nervous system, transfected cells were used for the assays. The use of chimeric $G\alpha_{qo5}$ (the last five amino acids of $G\alpha_o$ fused to $G\alpha_q$) provided a calcium flux assay suitable for higher throughput compound evaluation (Moreland *et al.,* 2004b). Further, using an agarose gel based method, live cells loaded with calcium indicator dyes and compounds spotted on a solid support, 8640 compounds per sheet could be assayed in a microarray compound screening format (Gopalakrishnan *et al.,* 2003). Certain promiscuous G proteins such as $G\alpha_{16}$ can lead to conflicting data with data obtained using naturally coupled receptors or receptors with chimeric G proteins (see Kostenis, 2001). Correlation with other functional assays is prudent.

Although *in vitro* assays focus on single channels, receptors or enzymes which are transfected and overexpressed into a host cell, the further challenge of peripheral and central (spinal and supraspinal) mechanisms leaves the investigator with the quandary of integrating the data from *in vitro* assays with the *in vivo* situation. Drug discovery has different assay requirements from basic research, since the goal is to identify novel, small molecules with a certain activity. Assays are performed in a high throughput manner to screen large numbers of compounds. One may detect an agonist or antagonist in an assay that optimizes detection but overestimates efficacy due to the number of receptors expressed or coupling system employed. This conundrum has been discussed recently including the advantages and disadvantages of using primary cultures of 'natural' cells (see Kenakin, 2003; 2004; Neubig *et al.,* 2003). The basic scientist using transfected cells and comparing to primary cultures of the organ system in question or tissue baths should be aware of this issue.

Ion channel assays

Ion channels (ligand gated and voltage gated channels) affect cellular ion flux, resulting in a variety of processes including smooth muscle contraction, neuronal firing and activation of endothelial signaling pathways (e.g. calcium induction of nitric oxide (NO) synthase). Ion channels play key roles in the LUT as well as in erectile function (see Christ, 2000; Gopalakrishnan & Shieh, 2004). The two major types of assays used to measure activity include flux assays (Gill *et al*., 2003) and electrophysiology measurements (e.g. Spektor *et al*., 2002). Flux assays can utilize radioactive ions (such as rubidium for the sodium, potassium ATPase, Gupta *et al.,* 1995) or calcium or sodium fluorescent indicators. Although electrophysiology is more labor intensive and focuses on single cells, this technique can elicit data unobtainable from other methodologies. Examples of these assays include characterization of Maxi K, K ATP, and Kv2.2 channels in corpus cavernosum (Lee *et al.,* 1999; Malysz *et al.,* 2002; Spektor *et al*., 2002).

Enzyme based assays

The NO pathway is key in vascular smooth muscle relaxation in penile erection (see Andersson, 2001; Moreland *et al*., 2001; 2004a). NO synthase assays measure the conversion of radiolabeled arginine to citrulline in tissue extracts (Kim *et al.,* 1993) as well as enable direct detection of NO (Becker *et al.,* 2000). NO activates soluble guanylate cyclase (sGC), increasing the synthesis of cGMP in the smooth muscle cell (Figure 1) (see Brioni *et al.,* 2002). Typically, these assays use recombinant sGC or tissue extracts and measure levels of cGMP by ELISA, RIA or enzymatic means. Allosteric activators of sGC have been described and enzyme kinetic assays have been used to elucidate these properties (Nakane *et al.,* 2002; Miller *et al.,* 2003). The effects of oxygen tension on prostaglandin synthesis in human corpus cavernosum smooth muscle cells and in rabbit corpus cavernosum have been determined. These assays utilize extraction and detection of the prostaglandin or prostaglandin metabolite by ELISA (Daley *et al.,* 1996; Moreland *et al.,* 2001).

Phosphodiesterases and phosphatases catalyze hydrolysis of cyclic nucleotides and phosphorylated substrates, respectively (see Wheeler *et al.,* 2005). Phosphodiesterase 5 (PDE5) inhibitors were recently discovered and used as oral first line therapeutics in the treatment of MEDs (Moreland *et al*., 2001, 2004a). The discussion of competitive and allosteric enzymatic inhibition is beyond the scope of this review and the reader is referred to other sources (see Neubig *et al.,* 2003; Kenakin, 2004). PDE5 has been assayed in extracts from corpus cavernosum, cultured corpus cavernosum smooth muscle cells, as well as platelets (Boolell *et al.,* 1996; Ballard *et al*., 1998; Moreland *et al.,* 1998). In these assays, radioactive cGMP is hydrolyzed and the remaining substrate quantitated after a period of time using ion exchange chromatography and scintillation counting. Data analysis has included the use of Dixon plots and determining inhibition at multiple substrate concentrations to determine the inhibition constant (K_i) (Ballard *et al*., 1998; Moreland *et al*., 1998). Similar assays can be carried out for other phosphodiesterases.

Tissue based assays, organ baths

Tissue bath studies allow the *ex vivo* study of muscle contraction in a system with more than one cell type. Information can be obtained regarding some neural responses (e.g. electrical stimulation and release of NO; Kim *et al*., 1993) as well as contractile and relaxatory processes in the target tissue. For studies on human MED, sources of tissues have relied on human surgical biopsies of corpus cavernosum obtained at the time of penile prosthesis insertion in men with MED or from penile cancer patients undergoing partial penectomy. The restricted availability of human penile tissues has led to use of cavernosal tissues isolated from rabbit as a major *in vitro* model. Similar information has been obtained by using tissues from mouse, rat, dog, horse and pig (see Andersson & Wagner, 1995, Giuliano *et al.,* 1999). The advantages of this method include ease in preparation, minimal equipment requirements and the fact that reproducible concentration–effect curves can be obtained using either contractile or relaxant agents. The response to pharmacological stimulation by adding phenylephrine, potassium chloride, or other contractile agents is the most common method used to contract the corpus cavernosum tissues *in vitro* to assess potency and efficacy of relaxing agents. Smooth muscle strip contractions in organ bath preparations can also be achieved by electric field stimulation similar to that employed to study neural responses (Kim *et al.,* 1993). Organ bath studies have been used to characterize peripheral α_1- (Traish *et al.,* 1995a, c) and α_2-adrenoceptors in rabbit and human corpus cavernosum (Traish *et al.,* 1997b; Gupta *et al.,* 1998) as well as define prostaglandin D receptors (Moreland *et al.,* 2002) and prostaglandin E receptors (Angulo *et al.,* 2002; Moreland *et al.,* 2003). These studies are amenable to classical pharmacology such as competition of antagonists (Schild analysis, Gupta *et al.,* 1998) and can provide a bridge between single cell and *in vivo* models.

Concluding remarks

A comprehensive review of pharmacologic *in vitro* assays even as they relate to LUT is beyond the scope of this review. It is hoped that the reader can benefit from our experiences and use these as a starting point for research on the LUT. One final comment concerns the differences between drug discovery and basic science effort. The goal of a drug discovery effort is to generate novel, highly selective molecules that either inhibit or activate processes in the cell to ultimately produce therapeutic effects in humans (see Pritchard *et al.,* 2003). Basic science provides a key role in this process by the discovery of new targets and physiologic processes. In drug discovery, *in vitro* models are key in the characterization of small molecules. While there have been a number of advances in defining the physiology and pharmacology of the LUT, recently discovered, selective tools provide opportunities to enhance our understanding of the LUT through both *in vitro* and *in vivo* approaches. The future is bright since many small molecule modulators of potential drug targets are available to provide proof of principle and hopefully to be ultimately evaluated in early phase clinical trials.

The author gratefully acknowledges a critical reading of this manuscript by Drs Connie Faltynek and Pamela Puttfarken in Neuroscience Research at Abbott Laboratories. He also thanks Karen M. Miner, R.N., for valuable editorial assistance. Apologies are offered in advance to all the authors of work not cited. This was not intended to be an exhaustive review but a starting point for future exploration.

References

ANDERSSON, K.E. (2001). Pharmacology of penile erection. *Pharmacol. Rev.,* **53,** 417–450.

ANDERSSON, K.E. & WAGNER, G. (1995). Physiology of penile erection. *Physiol. Rev.,* **75,** 191–236.

ANGULO, J., CUEVAS, P., LA FUENTE, J.M., POMEROL, J.M., RUIZ-CASTANE, E., PUIGVERT, A., GABANCHO, S., FERNANDEZ, A., NEY, P. & SAENZ DE TEJADA, I. (2002). Regulation of human penile smooth muscle tone by prostanoid receptors. *Br. J. Pharmacol.,* **136,** 23–30.

BALLARD, S.A., GINGELL, C.J., TANG, K., TURNER, L.A., PRICE, M.E. & NAYLOR, A.M. (1998). Effects of sildenafil on the relaxation of human corpus cavernosum tissue *in vitro* and on the activities of cyclic nucleotide phosphodiesterase isozymes. *J. Urol.,* **159,** 2164–2171.

BASKIN, L.S., HOWARD, P.S., DUCKETT, J.W., SNYDER, H.M. & MACARAK, E.J. (1993). Bladder smooth muscle cells in culture: I. Identification and characterization. *J. Urol.,* **149,** 190–197.

BECKER, A.J., UCKERT, S., TSIKAS, D., NOACK, H., STIEF, C.G., FROLICH, J.C., WOLF, G. & JONAS, U. (2000). Determination of nitric oxide metabolites by means of the Griess assay and gas chromatography-mass spectrometry in the cavernous and systemic blood of healthy males and patients with erectile dysfunction during different functional conditions of the penis. *Urol. Res.,* **28,** 364–369.

BOOLELL, M., ALLEN, M.J., BALLARD, S.A., GEPI-ATTEE, S., MUIRHEAD, G.J., NAYLOR, A.M., OSTERLOH, I.H. & GINGELL, C. (1996). Sildenafil: an orally active type 5 cyclic GMP-specific phosphodiesterase inhibitor for the treatment of penile erectile dysfunction. *Int. J. Impot. Res.,* **8,** 47–52.

BRIONI, J.D., MORELAND, R.B., COWART, M., HSIEH, G.C., STEWART, A.O., HEDLUND, P., DONNELLY-ROBERTS, D., NAKANE, M., LYNCH, J.J., KOLASA, T., POWLAKOSKI, J., OSINSKI, M., MARSH, K.C, ANDERSSON, K.-E. & SULLIVAN, J.P. (2004). Activation of dopamine D_4 receptors by ABT-724 facilitates penile erection in rats. *Proc. Natl. Acad. Sci. U.S.A.,* **101,** 6758–6763.

BRIONI, J.D., NAKANE, M., HSIEH, G., MORELAND, R.B., KOLASA, T. & SULLIVAN, J.P. (2002). Activators of soluble guanylate cyclase for the treatment of male erectile dysfunction. *Int. J. Impot. Res.,* **14,** 8–14.

CHRIST, G.J. (2000). Gap junctions and ion channels: relevance to erectile dysfunction. *Int. J. Impot. Res.,* **12** (Suppl 4), S15–S25.

CORVIN, S., STRASSER, H., BOESCH, S.T., BARTSCH, G. & KLOCKER, H. (2001). Human rhabdosphincter cell culture: a model for videomicroscopy of cell contractions. *Prostate,* **47,** 189–193.

DALEY, J.T., BROWN, M.L., WATKINS, M.T., TRAISH, A.M., HUANG, Y.H., MORELAND, R.B. & SAENZ DE TEJADA, I. (1996). Prostanoid production in rabbit corpus cavernosum. I. Regulation by oxygen tension. *J. Urol.,* **155,** 1482–1487.

DEGROAT, W.C. & YOSHIMURA, N. (2001). Pharmacology of the lower urinary tract. *Ann. Rev. Pharmacol. Toxicol.,* **41,** 691–721.

DE VRY, J., SCHOHE-LOOP, R., HEINE, H.G., GREUEL, J.M., MAULER, F., SCHMIDT, B., SOMMERMEYER, H. & GLASER, T. (1998). Characterization of the aminomethylchroman derivative BAY x 3702 as a highly potent 5-hydroxytryptamine1A receptor agonist. *J. Pharmacol. Exp. Ther.,* **284,** 1082–1094.

EYSTER, KM. (1998). Introduction to signal transduction: a primer for untangling the web of intracellular messengers. *Biochem. Pharmacol.,* **55,** 1927–1938.

GILL, S., GILL, R., LEE, S.S., HESKETH, J.C., FEDIDA, D., REZAZADEH, S., STANKOVICH, L. & LIANG, D. (2003). Flux assays in high throughput screening of ion channels in drug discovery. *Assay Drug Dev. Technol.,* **1,** 709–717.

GIULIANO, F., RAMPIN, O. & MCKENNA, K.E. (1999). Animal models used in erectile dysfunction. In: *Textbook of Erectile Dysfunction,* eds. Carson, C.C., Kirby, R. & Goldstein, I., pp. 43–49. Oxford: ISIS Medical Media.

GOPALAKRISHNAN, M. & SHIEH, c.c. (2004). Potassium channel subtypes as molecular targets for overactive bladder and other urological disorders. *Expert Opin. Ther. Targets,* **8,** 437–458.

GOPALAKRISHNAN, S.M., MORELAND, R.B., KOFRON, J.L., HELFRICH, R.J., GUBBINS, E., MCGOWEN, J., MASTERS, J.N., DONNELLY-ROBERTS, D., BRIONI, J.D., BURNS, D.J. & WARRIOR, U. (2003). A cell-based micro arrayed compound screening format for identifying modulators of G-protein coupled receptors: application for pharmacological studies and high throughput screening. *Anal. Biochem.,* **321,** 192–201.

GUPTA, S., MORELAND, R.B., DALEY, J.T., YANG, S., GOLDSTEIN, I. & TRAISH, A.M. (1998). Corpus cavernosum smooth muscle expresses functional post-synaptic alpha-2-adrenergic receptors. *Br. J. Pharmacol.,* **123,** 1237–1245.

GUPTA, S., MORELAND, R.B., MUÑARRIZ, R. & SAENZ DE TEJADA, I. (1995). Possible role of sodium potassium ATPase in the regulation of human corpus cavernosum smooth muscle contractility by nitric oxide. *Br. J. Pharm.,* **116,** 2201–2206.

HOROWITZ, A., MENICE, C.B., LAPORTE, R. & MORGAN, K.R. (1996). Mechanisms of smooth muscle contraction. *Physiol. Rev.,* **76,** 967–1003.

JESSANI, N. & CRAVATT, B.F. (2004). The development and application of methods for activity-based protein profiling. *Curr. Opin. Chem. Biol.,* **8,** 54–59.

JESSANI, N., NIESSEN S WEI, B.Q., NICOLAU, M., HUMPHREY, M., JI, Y., HAN, W., NOH, D.Y., YATES, J.R., JEFFREY, S.S. & CRAVATT, B.F. (2005). A streamlined platform for high-content functional proteomics of primary human specimens. *Nat. Methods,* **2,** 691–697.

KENAKIN, T. (2003). Predicting therapeutic value in the lead optimization phase of drug discovery. *Nature Rev Drug Discovery,* **2,** 429–438.

KENAKIN, T. (2004). Principles: receptor theory in pharmacology. *Trends Pharmacol. Sci.,* **25,** 186–192.

KIM, N., VARDI, Y., PADMA-NATHAN, H., DALEY, J., GOLDSTEIN, I., SAENZ, D.E. & TEJADA, I. (1993). Oxygen tension regulates the nitric oxide pathway. Physiological role in penile erection. *J. Clin. Invest.,* **91,** 437–442.

KOSTENIS, E. (2001). Is Galpha 16 the optimal tool for fishing ligands of orphan G-protein-coupled receptors? *Trends Pharmacol. Sci.,* **22,** 560–564.

KROPP, B.P., ZHANG, Y., TOMASEK, J.J., COWAN, R., FURNESS, P.D., VAUGHAN, M.B., PARIZI, M. & CHENG, E.Y. (1999). Characterization of cultured bladder smooth muscle cells: assessment of *in vitro* contractility. *J. Urol.,* **162,** 1779–1784.

LEE, S.W., WANG, H.Z. & CHRIST, G.J. (1999). Characterization of ATP-sensitive potassium channels in human corporal smooth muscle cells. *Int. J. Impot. Res.,* **11,** 179–188.

LEVIN, R.M., HAUGAARD, N., LEVIN, S.S., BUTTYAN, R., CHEN, M.W., MONSON, F.C. & WEIN, A.J. (1995). Bladder function in experimental outlet obstruction: pharmacologic responses to alterations in innervation, energetics, calcium mobilization, and genetics. *Adv. Exp. Med. Biol.,* **385,** 7–19.

MALYSZ, J., FARRUGIA, G., OU, Y., SZURSZEWSKI, J.H., NEHRA, A. & GIBBONS, S.J. (2002). The Kv2.2 alpha subunit contributes to delayed rectifier K(+) currents in myocytes from rabbit corpus cavernosum. *J. Androl.,* **23,** 899–910.

MILLER, L.N., NAKANE, M., HSIEH, G.C., KOLASA, T., MORELAND, R.B. & BRIONI, J.D. (2003). A-350619: a novel activator of soluble guanylate cyclase. *Life Sci.,* **72,** 1015–1025.

MORELAND, R.B., ALBADAWI, H., BRATTON, C., PATTON, G., GOLDSTEIN, I., TRAISH, A.M. & WATKINS, M.T. (2001). Oxygen-dependent prostanoid synthesis activates functional PGE receptors on human corpus cavernosum smooth muscle cells. *Am. J. Physiol.,* **281,** H552–H557.

MORELAND, R.B., BRIONI, J.D. & SULLIVAN, J.P. (2004a). Emerging pharmacologic approaches for the treatment of lower urinary tract disorders. *J. Pharmacol. Exp. Ther.,* **308,** 797–804.

MORELAND, R.B., GOLDSTEIN, I. & TRAISH, A. (1998). Sildenafil, a novel inhibitor of phosphodiesterase type 5 in human corpus cavernosum smooth muscle cells. *Life Sci.,* **62,** 309–318.

MORELAND, R.B., HSIEH G NAKANE, M. & BRIONI, J. (2001). The biochemical and neurologic basis for treatment of male erectile dysfunction. *J. Pharm. Exp. Ther.,* **296,** 225–234.

MORELAND, R.B., KIM, N.N., NEHRA, A., GOLDSTEIN, I. & TRAISH, A.M. (2003). Expression of functional prostaglandin E (EP) receptors in human corpus cavernosum smooth muscle: Implications for PGE_1 action. *Int. J. Impot. Res.,* **15,** 362–368.

MORELAND, R.B., NAKANE, M., DONNELLY ROBERTS, D., MILLER, L., CHANG, R., UCHIC, M., TERRANOVA, M.A., GUBBINS, E., HELFRICH, R., NAMOVIC, M., EL-KOUHEN, O., MASTERS, J.N. & BRIONI, J.D. (2004b). Comparative pharmacology of human dopamine D_2-like receptor stable cell lines coupled to calcium flux through $G\alpha_{qo}$. *Biochem. Pharmacol.,* **68,** 761–772.

MORELAND, R.B., NEHRA, A., KIM, N.N., MIN, K.-S., ALBADAWI, H., WATKINS, M.T., GOLDSTEIN, I. & TRAISH, A.M. (2002). Expression of functional prostaglandin D (DP) receptors in human corpus cavernosum smooth muscle. *Int. J. Impot. Res.,* **14,** 446–452.

MORELAND, R.B., TERRANOVA, M.A., CHANG, R., UCHIC, M., MATULENKO, M., SURBER, B., STEWART, A.O. & BRIONI, J.D. (2004c). [^{3}H] A-369508 ([2-[4-(2-cyanophenyl)-1-piperazinyl]-*N*-(3-methylphenyl) acetamide): an agonist radioligand selective for the dopamine D_4 receptor. *Eur. J. Pharmacol.,* **497,** 147–154.

NAKANE, M., HSIEH, G., MILLER, L.N., CHANG, R., TERRANOVA, M.A., MORELAND, R.B., KOLASA, T. & BRIONI, J.D. (2002). Activation of soluble guanylate cyclase causes relaxation of corpus cavernosum tissue: synergism of nitric oxide and YC-1. *Int. J. Impot. Res.,* **14,** 121–127.

NEUBIG, R.R., SPEDDING, M., KENAKIN, T. & CHRISTOPOULOS, A. (2003). International Union of Pharmacology Committee on Receptor Nomenclature and Drug Classification. XXVIII. Update on terms and symbols in quantitative pharmacology. *Pharm. Rev.,* **55,** 597–606.

NEWMAN-TANCREDI, A., CUSSAC, D., AUDINOT, V., NICOLAS, J.P., DE CEUNINCK, F., BOUTIN, J.A. & MILLAN, M.J. (2002). Differential actions of antiparkinson agents at multiple classes of monoaminergic receptor. II. Agonist and antagonist properties at subtypes of dopamine D_2-like receptor and $alpha_1$/$alpha_2$-adrenoceptor. *J. Pharmacol. Exp. Ther.,* **303,** 805–814.

PONTARI, M.A., BRAVERMAN, A.S. & RUGGIERI, M.R. (2004). The M2 muscarinic receptor mediates *in vitro* bladder contractions from patients with neurogenic bladder dysfunction. *Am. J. Physiol.,* **286,** R874–R880.

PRITCHARD, J.F., JURIMA-ROMET, M., REIMER, M.L., MORTIMER, E., ROLFE, B. & CAYEN, M.N. (2003). Making better drugs: decision gates in non-clinical drug development. *Nat. Rev. Drug Discov.,* **2,** 542–553.

SPEKTOR, M., RODRIGUEZ, R., ROSENBAUM, R.S., WANG, H.Z., MELMAN, A. & CHRIST, G.J. (2002). Potassium channels and human corporeal smooth muscle cell tone: further evidence of the physiological relevance of the Maxi-K channel subtype to the regulation of human corporeal smooth muscle tone *in vitro*. *J. Urol.,* **167,** 2628–2635.

SU, X., CHANGOLKAR, A., CHACKO, S. & MORELAND, R.S. (2004). Diabetes decreases rabbit bladder smooth muscle contraction while increasing levels of myosin light chain phosphorylation. *Am. J. Physiol.,* **287,** F690–F699.

TRAISH, A.M., MORELAND, R.B., GALLANT, C., HUANG, Y.-H. & GOLDSTEIN, I. (1997a). G-protein-coupled receptor agonists augment adenylyl cyclase activity induced by forskolin in human corpus cavernosum smooth muscle cells. *Receptors Signal Transduct.,* **7,** 123–134.

TRAISH, A.M., MORELAND, R.B., HUANG, Y.-H. & GOLDSTEIN, I. (1997b). Expression of functional alpha-2-adrenergic receptor subtypes in human corpus cavernosum and in human corpus cavernosum smooth muscle cells. *Receptors Signal Transduct.,* **7,** 55–67.

TRAISH, A.M., NETSUWAN, N., DALEY, J., PADMAN-NATHAN, H., GOLDSTEIN, I. & SAENZ DE TEJADA, I. (1995a). A heterogeneous population of alpha 1 adrenergic receptors mediates contraction of human corpus cavernosum smooth muscle to norepinephrine. *J. Urol.,* **153,** 222–227.

TRAISH, A.M., PALMER, M.A., GOLDSTEIN, I. & MORELAND, R.B. (1995b). Expression of functional muscarinic acetylcholine receptor subtypes in cultured human corpus cavernosum and in cultured smooth muscle cells. *Receptors,* **5,** 159–176.

TRAISH, A.M., TOSELLI, P., GUPTA, S., SAENZ DE TEJADA, I., GOLDSTEIN, I. & MORELAND, R.B. (1995c). Identification of alpha 1-adrenergic receptor subtypes in human corpus cavernosum tissue and in cultured trabecular smooth muscle cells. *Receptor,* **5,** 145–157.

WHEELER, M.A., AYYAGARI, R.R., WHEELER, G.L. & WEISS, R.M. (2005). Regulation of cyclic nucleotides in the urinary tract. *J. Smooth Muscle Res.,* **41,** 1–21.