Management of Erectile Function by Penile Purinergic P2 Receptors in the Diabetic Rat

Serap Gur, Philip J. Kadowitz, Asim B. Abdel-Mageed, Muammer Kendirci, Suresh C. Sikka, Geoffrey Burnstock and Wayne J. G. Hellstrom*

From the Departments of Urology and Pharmacology, Tulane Health Sciences Center, New Orleans, Louisiana, and Autonomic Neuroscience Institute, Royal Free and University College Medical School (GB), London, United Kingdom

Purpose: We determined the role of purine and pyrimidine nucleotides in erectile function in diabetic rats.

Materials and Methods: A total of 60 adult male rats were divided into 2 groups, including 30 controls and 30 treated with streptozotocin (60 mg/kg) for 8 weeks to induce hyperglycemia. Changes in intracavernous pressure after intracrural injections of adenosine 5′triphosphate and adenosine 5′triphosphate analogues in control and diabetic rats, and the relaxant response to electrical field stimulation of precontracted corpus cavernosum smooth muscle in organ baths were investigated. The localization of P2X1, P2Y1 and P2Y2 receptors was assessed in penile tissue via an immunohistochemical approach.

Results: Corpus cavernosum smooth muscle relaxation in vivo and by electrical field stimulation in vitro was significantly decreased in diabetic rats. Adenosine 5′triphosphate (P2X, P2Y), 2-methylthioadenosine 5′triphosphate (P2Y1) and uridine 5′-triphosphate (P2Y2) agonists but not α,β-methylene adenosine 5′triphosphate (a P2X1 agonist) significantly improved the erectile response to electrical field stimulation in diabetic rat corpus cavernosum smooth muscle. Although intracavernous pressure/mean arterial pressure values in the rats were not restored in the presence of the P2X1 antagonist PPADS, the relaxation response to electrical field stimulation in isolated corpus cavernosum smooth muscle from diabetic rats was improved. Abundant immunoreactivity for PX1 and P2Y2 receptors was observed in penile tissues from diabetic rats compared to that from control rats.

Conclusions: These results demonstrate 1) heterogeneous effects of purinergic agonists on corporeal function in diabetic rats, and 2) the activation of P2Y1 and P2Y2 receptor relaxation of corpus cavernosum smooth muscle to induce erection in rats and perhaps improve erectile function in men with diabetes.

Key Words: penis; erectile dysfunction; diabetes mellitus; receptors, purinergic; rats, Sprague-Dawley

Abbreviations and Acronyms

2-MeSATP = 2-methylthioATP

α,β-MeATP = α,β-methylene ATP

ATP = adenosine 5′triphosphate

BSA = bovine serum albumin

CCSM = corpus cavernosum smooth muscle

EC = endothelial cell

ED = erectile dysfunction

EFS = electrical field stimulation

ICP = intracavernous pressure

MAP = mean arterial pressure

NANC = nonadrenergic noncholinergic

NO = nitric oxide

PE = polyethylene

PKC = protein kinase C

PPADS = pyridoxal-phosphate-6-azophenyl-2′, 4′-disulfonate

RB2 = reactive blue-2

STZ = streptozotocin

UTP = uridine 5′-triphosphate

Submitted for publication July 9, 2008.

Study received Tulane University animal care and use committee approval.

* Correspondence: Section of Andrology, Department of Urology, Tulane University Health Sciences Center, 1430 Tulane Ave., SL-42, New Orleans, Louisiana 70112 (telephone: 504-988-7308; FAX: 504- 988-5059; e-mail: whellst@tulane.edu).

NUCLEOTIDES such as ATP and UTP modulate cellular function by activating membrane bound P2 receptors. These receptors have been divided into P2X (mainly excitatory) and P2Y (mainly inhibitory) types.[1,2] Seven subtypes of P2X receptors and 8 subtypes of P2Y receptors have been characterized.[1,3]

ATP is a NANC neurotransmitter that innervates CCSM.[4] Takahashi et al reported that intracavernous injection of ATP increased ICP and induced erection in dogs.[5] In 2000 Lee

0022-5347/09/1815-2375/0
THE JOURNAL OF UROLOGY®

Vol. 181, 2375-2382, May 2009
Printed in U.S.A.
DOI:10.1016/j.juro.2009.01.002

et al noted the presence of immunoreactive P2X1 and P2X2 receptors in CCSM and penile blood vessels.[6] Obara et al documented P2Y1 receptor expression in endothelial cells that line the lacunar spaces and in penile blood vessels.[7] The precise role of purinergic receptor function during erection is still not fully delineated.[8]

ED is highly prevalent in men with diabetes with an approximately 50% rate at age 50 years.[9] Since NO has a dominant role in erectile function, most researchers propose that diabetes related ED is a result of impaired NANC nerve function and dysfunctional endothelium in CCSM.[9] Saenz de Tejada et al observed that in CCSM strips from patients with diabetes and ED neurogenic and endothelium dependent relaxation responses are decreased.[9]

Since NANC nerves release nucleotide transmitters and NANC function is impaired in patients with diabetes, we hypothesized that administering P2X and P2Y receptor analogues would improve erectile function in diabetic rats.[10,11] Thus, we determined 1) the role of ATP, UTP and other purine analogues for potentiating EFS induced relaxation responses in isolated CCSM and 2) which purinoceptor subtypes are involved in CCSM responses to EFS.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats weighing 300 to 325 gm were divided into 1) controls and 2) diabetic rats treated with STZ for 8 weeks. Diabetes was induced by an injection of STZ (60 mg/kg intraperitoneally) dissolved in citrate buffer (pH 7.0). Control rats received an injection of citrate buffer alone. All protocols were approved by the Tulane University animal care and use committee.

Physiological Erection Studies

The rats were anesthetized with pentobarbital (50 mg/kg intraperitoneally). The trachea was cannulated with PE-240 tubing to maintain a potent airway. The left carotid artery was cannulated with PE-50 tubing. A 25 gauge needle filled with 250 U/ml heparin and connected to PE-50 tubing was inserted into the right crura. The right major pelvic ganglion and cavernous nerve were identified and a stainless steel bipolar hook electrode was placed around the cavernous nerve. The cavernous nerve was stimulated with a square pulse stimulator at 2.5, 5 and 7.5 V at 15 Hz for 30 seconds. MAP and ICP were measured with Statham P23 pressure transducers connected to an MP100 data acquisition system (Biopac Systems, Goleta, California).

The erectile response was measured as the change in ICP/MAP in control and diabetic rats following administration of the agonists ATP (100 μM), UTP (100 μM), α,β-methylene ATP (1 μM) and 2-MeSATP (100 μM) as well as the purinergic antagonists PPADS (30 μM) and/or RB2 (100 μM). Intracrural administration of drugs dissolved in normal saline was delivered in a small volume (10 μl) to avoid volume related changes in ICP. ICP and MAP were measured at baseline and continuously after the injection of each agent.

In Vitro Measurement of Isometric Force Generation in Tissue Strips

Rat CCSM strips ($1 \times 1 \times 5$ mm) were placed in an organ bath containing Krebs solution, composed of 118 mM NaCl, 4.7 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 25 mM $NaHCO_3$, 2.5 mM $CaCl_2$ and 10 mM glucose, and equilibrated with 95% O_2-5% CO_2 at 37C under 1 gm tension. EFS was applied to parallel platinum electrodes on either side of the CCSM strips for 15 seconds at 100 V and 1 to 20 Hz using an S48 electronic stimulator (Grass Instruments, Quincy, Massachusetts). After the CCSM strips were contracted to a steady state with phenylephrine the relaxation induced by EFS was measured at baseline and then repeated in the presence of each agonist, including ATP (100 μM), UTP (100 μM), α,β-MeATP (1 μM) and 2-MeSATP (100 μM), and/or antagonists, including PPADS (30 μM) and/or RB2 (100 μM), which were added to the organ bath 1 minute before applying EFS.

Immunohistochemistry

Rat corpus cavernous tissues were sectioned at 12 μm using a cryostat and placed on gelatin coated slides. Sections were treated with 50% methanol containing 0.4% hydrogen peroxide for 10 minutes. Nonspecific protein binding sites were blocked with 10% normal horse serum (diluted 1:50) in phosphate buffered saline containing 0.1% (weight per volume) bovine serum albumin. Slides were incubated with rabbit primary polyclonal antibody (diluted 1:200) in 10% normal horse serum for 1 hour at room temperature. Samples were washed and incubated an additional 30 minutes with biotinylated secondary antibody, followed by a further 30-minute incubation with avidin-biotin-conjugated horseradish peroxidase (Dako, Carpinteria, California) and then the substrate diaminobenzidine (Vectastain®) for 5 minutes. Harris hematoxylin was used as a counterstain and negative control slides were stained with secondary antibody only. Images were visualized under light microscopy using a DM4000B microscope and DFC 280 color digital camera system (Leica®).

Data Analysis and Statistics

In vivo erectile responses are expressed as the ratio of ICP in mm Hg to MAP in mm Hg. Isometric force generation data were measured in mg or as a percent of the maximal change with phenylephrine (10 μM) induced force generation considered as 100%. All data are presented as the mean $\pm$ SEM. Statistical differences were determined by ANOVA, followed by Bonferroni's multiple comparison test using Prism® 4 with $p < 0.05$ considered significant.

RESULTS

Body and Corpus Cavernosum Weight, and Blood Glucose Levels

STZ treated diabetic rats had significant weight loss compared to control rats (mean 279.1 $\pm$ 16.7 vs

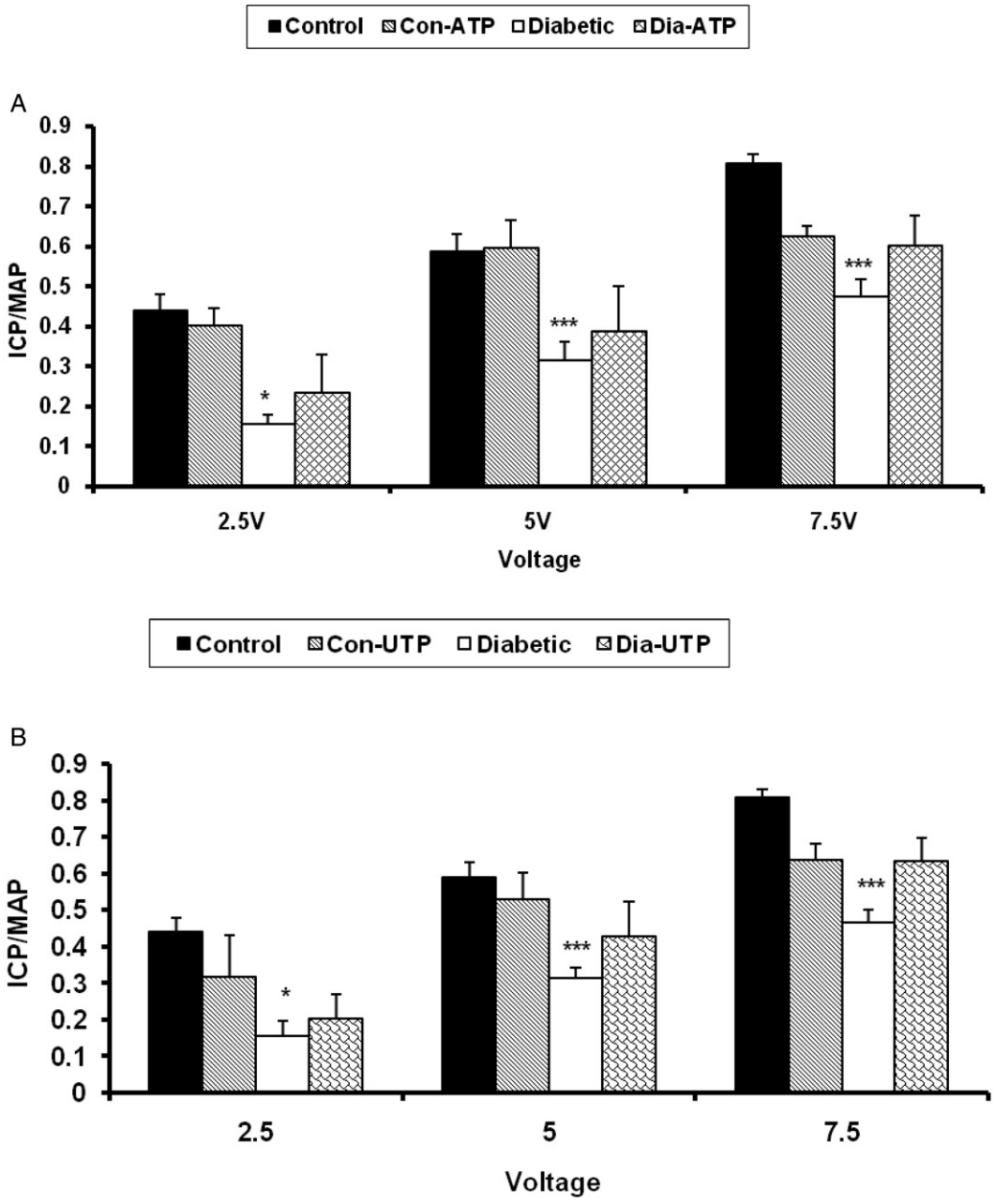

Figure 1. Alterations in ICP/MAP in response to direct intracrural injection of ATP (*A*), and UTP (*B*) values in control (*Con*) and diabetic (*Dia*) rats. Asterisks indicate significantly different response. Single asterisk indicates $p < 0.05$ vs controls. Triple asterisks indicate $p < 0.001$ vs controls.

471.3 ± 10.9 gm, $p < 0.001$). Blood glucose levels were significantly higher in the diabetic group than in the control group (509.3 ± 22.9 vs 94.13 ± 4.7 mg/dl, $p < 0.001$). CCSM strip weights were not significantly different in control and diabetic rats (0.0309 ± 0.004 vs 0.020 ± 0.0037 gm, $p = 0.0628$).

Effects of Purinergic Agents on In Vivo Erectile Responses

ICP/MAP values at all voltage settings were not significantly changed after ATP (100 μM) or UTP (100 μM) administration in control rats (fig. 1). The decrease in ICP/MAP values at all voltage settings in diabetic rats was normalized after intracrural administration of ATP or UTP compared to that in control rats. When α,β-MeATP was given, ICP/MAP values were decreased in control but not in diabetic rats ($p < 0.05$, fig. 2). In the presence of the P2X1 agonist α,β-MeATP (1 μM) the P2X1 antagonist PPADS (30 μM) appeared not to have resulted in normalized erectile function in control and diabetic rats (fig. 2). In addition, the P2Y1 receptor agonist 2-MeSATP (100 μM) did not result in significant

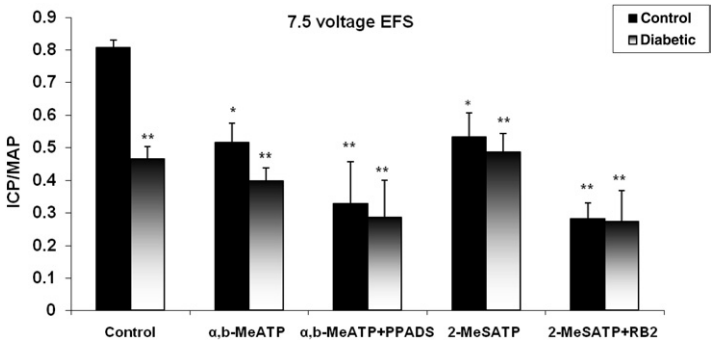

Figure 2. Alterations in ICP/MAP in response to cavernous nerve stimulation and direct left intracrural injection of α,β-MeATP with or without PPADS and 2-MeSATP with or without RB2 in control and diabetic rats. Data are shown as mean ± SEM. Single asterisk indicates $p < 0.05$ vs controls. Double asterisks indicate $p < 0.01$ vs controls.

improvement in the diabetic rat responses whether alone or in the presence of RB2 (fig. 2).

Effect of ATP and UTP on EFS Induced Relaxation Responses in CCSM Strips

EFS in phenylephrine precontracted isolated CCSM produced frequency dependent relaxation. EFS at 20 Hz caused a maximum relaxation of approximately 66%. Diabetes decreased this response by about 35%, which was reversed to control levels by the administration of ATP (100 μm) (fig. 3, *A*). EFS induced relaxation in the CCSM of diabetic rats was enhanced in the presence of UTP (100 μm) (fig. 3, *B*). Moreover, this enhancement was 27% greater than control values.

Effect of P2 Agonists and Antagonists on EFS Induced Relaxation in CCSM Strips

EFS induced relaxation of CCSM strips from control rats at 20 Hz was decreased by the P2X1 agonist α,β-MeATP (1 μM), whereas the response in diabetic rats appeared to be unchanged (fig. 4). CCSM strips from control and diabetic rats showed a reversal of the decrease in EFS induced relaxation by the P2X antagonist PPADS and restored the response to control values. The P2Y1 receptor agonist 2-MeSATP alone completely restored the decreased EFS induced relaxation in diabetic rat CCSM strips. This EFS response was antagonized by RB2 partially in control rats and completely in diabetic rats (fig. 4).

Immunohistochemical Evidence of P2X1, P2Y1 and PY2 Receptors in Penile Tissues

Diabetic penile tissue showed intense localization of P2X1 receptors on endothelial cells in lacunar spaces and CCSM cells compared to that in control slides (fig. 5). P2Y1 receptors were localized to endothelial cells and CCSM in controls and there was no positive immunostaining in diabetic rats (fig. 6, *A* and *B*). While P2Y2 receptor expression was detected partly in CCSM cells in the control group, intense P2Y2 immunoreactivity was observed in CCSM in the diabetic group (fig. 6, *C* and *D*).

DISCUSSION

To our knowledge this is the first study to show that ATP and UTP can restore nerve mediated CCSM relaxation in the diabetic rat penis. In addition, these data reveal that the beneficial effect of ATP in the enhancement of neuronal responses in the diabetic rat penis is mediated by the activation of P2Y1 and P2Y2 receptors relative to P2X1 receptor activation. Evidence in support of these conclusions includes 1) enhanced erectile responses in vivo and enhanced EFS evoked neuronal response in vitro in CCSM strips from diabetic rats after exogenous ATP administration, 2) when P2X receptors were stimulated alone by the P2X1 agonist, α,β-meATP, the antagonistic effect of PPADS was clearly demonstrated, 3) P2Y1 and P2Y2 agonists reversed the decrease in function in the EFS induced neuronal response and 4) while P2X1 and P2Y2 receptors were expressed in CCSM, P2Y1 receptors were observed in endothelium and to a lesser extent in outer sinusoidal cells of the diabetic rat penis.

We observed that intracavernous ATP injection did not induce an erectile response in control rats. Previous experiments have shown that intracavernous ATP injection in the normal canine penis caused a dose dependent erectile response.[5,12] It is not clear at this time whether the difference in the response to ATP may be attributable to species variation. The current study documents that STZ induced hyperglycemia in rats induces a loss of erectile function and further supports ATP prevention of the decrease in erectile response, as shown by the EFS induced relaxation of CCSM in diabetic rats.[10,11] Earlier studies have revealed that intraperitoneal injection of adenine nucleotides induced a sustained increase in plasma ATP levels that lasted for hours after the

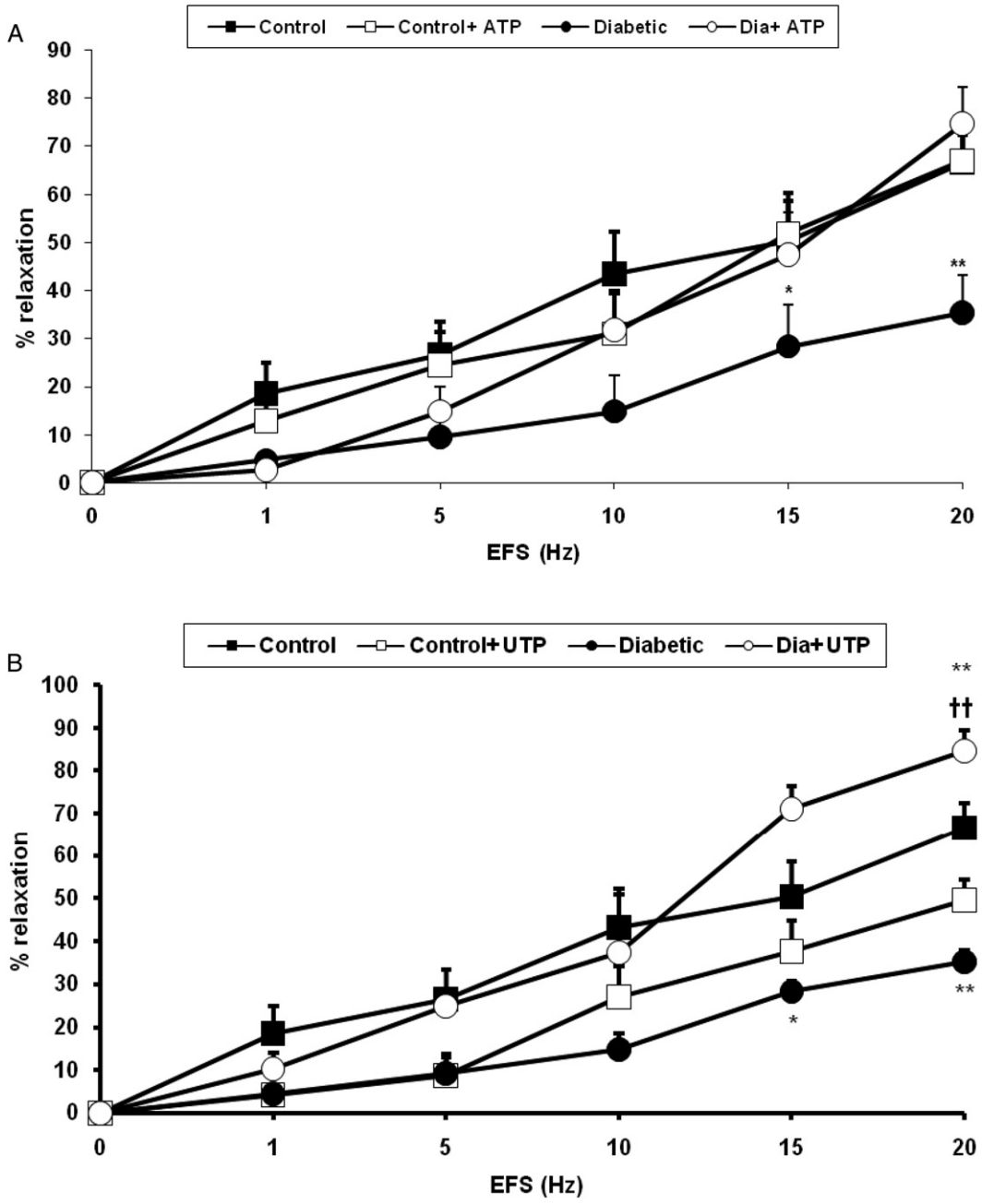

Figure 3. Effects of ATP (*A*) and UTP (*B*) on rat CCSM precontracted submaximally with 10 μM phenylephrine. There was significantly enhanced EFS induced relaxant responses in presence of these agonists in diabetic vs control CCSM strips. Single asterisk indicates $p < 0.05$ vs controls. Double asterisks indicate $p < 0.01$ vs controls. Double daggers indicate $p < 0.001$ for treatment effect vs controls.

initial injection.[13] In addition, a recent study showed a decreased response to the growth of prostate cancer by ATP injection.[14] Clinical trials in patients with cancer have established that the systemic administration of ATP is safe with the potential for benefit as an anticancer treatment.[15] In the current study ATP enhancement of EFS induced relaxation was shown to be mediated by interaction with ATP sensitive P2X and P2Y receptor subtypes. In addition, the P2X and P2Y receptor subtypes may exert opposing responses with the net effect of ATP being the algebraic summation of contractile and relaxant actions.

In in vivo studies in diabetic rats a single injection of the P2X1 agonist α,β-MeATP partially decreased the erectile response. In addition, PPADS significantly reversed the decrease in EFS induced relaxation in isolated CCSM from diabetic rats but not under in vivo conditions. Such differences may be attributable to disparate in vivo and in vitro

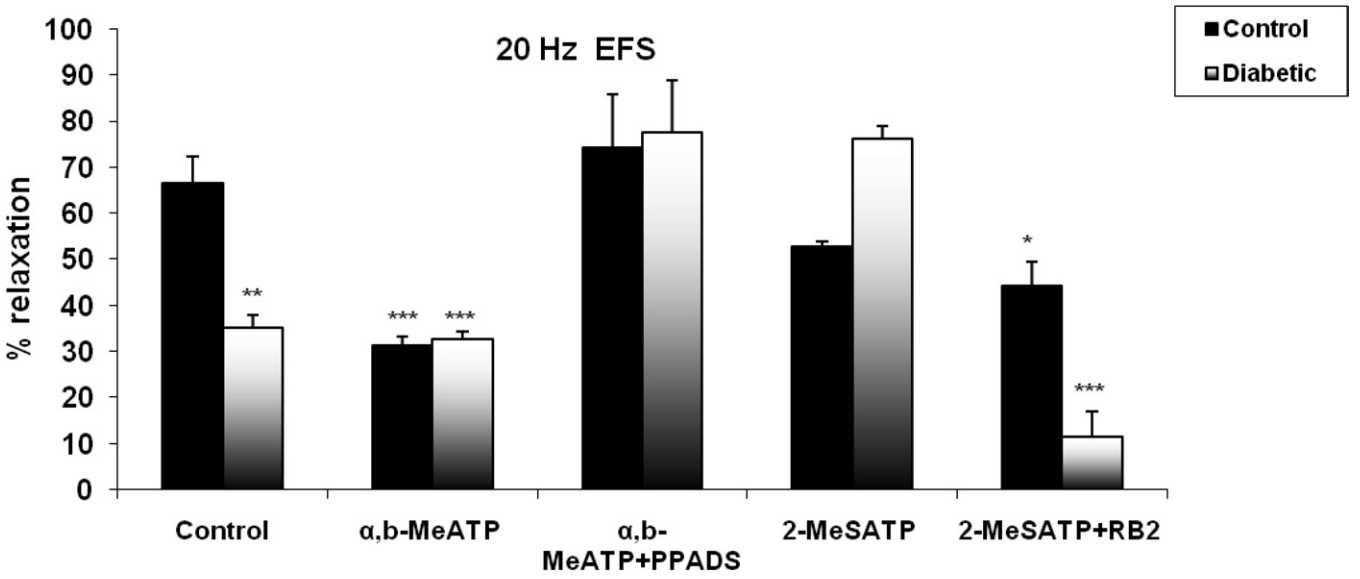

Figure 4. Effects of α,β-MeATP with or without PPADS and 2-MeSATP with or without RB2 on EFS induced relaxation in CCSM from control and diabetic rats precontracted with 10 μM phenylephrine. Data are shown as mean $\pm$ SEM. Single asterisk indicates $p < 0.05$ vs controls. Double asterisks indicate $p < 0.01$ vs controls. Triple asterisks indicate $p < 0.001$ vs controls.

conditions, the role of circulating unknown mediators, and/or hemodynamic factors in the intact penis and/or the various anesthetic agents used. The integrated hemodynamic response to P2 receptor activation in vivo is not well-defined. In addition, the activation of P2X receptor on peripheral nerve terminals and synergistic effects with other mediators can enhance the response of the peripheral nerve terminal to other stimuli.[16] We speculate that the occupation of P2X1 receptors by ATP leads to contraction while P2X1 receptors transmit the ionotropic responses of ATP. Other explanations may be related to PKC overactivity in diabetes, which is responsible for NO dependent vascular and autonomic nerve dysfunction.[17] It is possible that PKC induced phosphorylation of the ATP gated ion channels (P2X1 receptor) to change conductance of the channel.[18] This pharmacological evidence is further supported by the immunohistochemical demonstration of abundant P2X1 receptor expression in CCSM

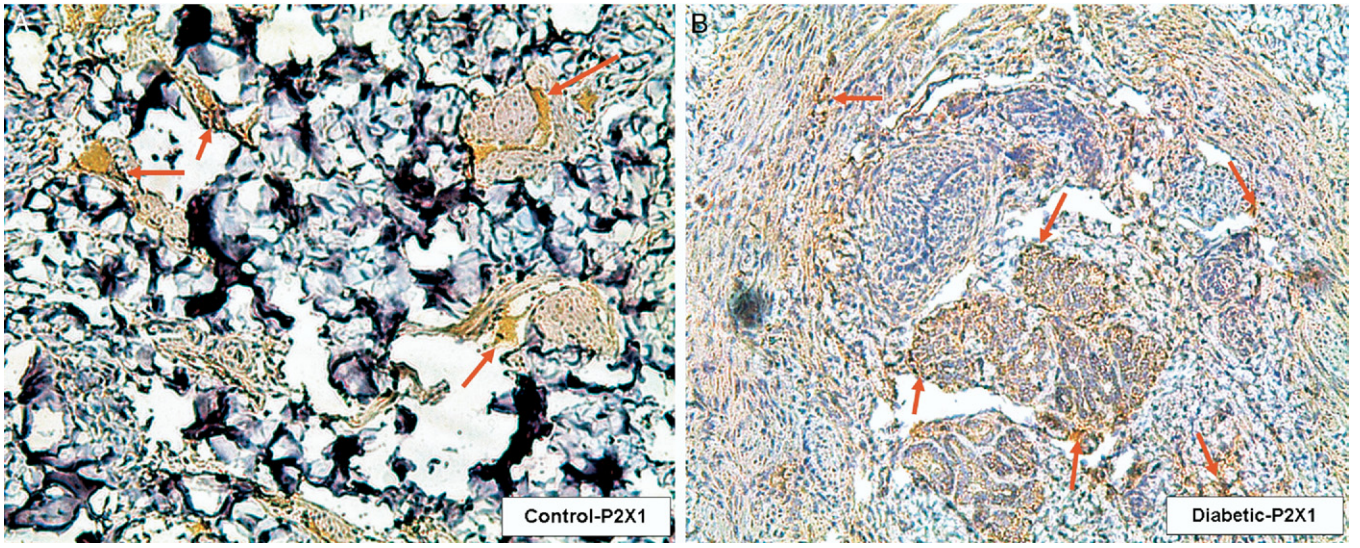

Figure 5. Representative immunohistochemical staining for P2X1 receptors in control (*A*) and diabetic (*B*) rat corpus cavernosum demonstrates abundant staining for P2X1 (arrows) in CCSM in diabetic group, as observed by 6 independent observers. Reduced from $\times$100.

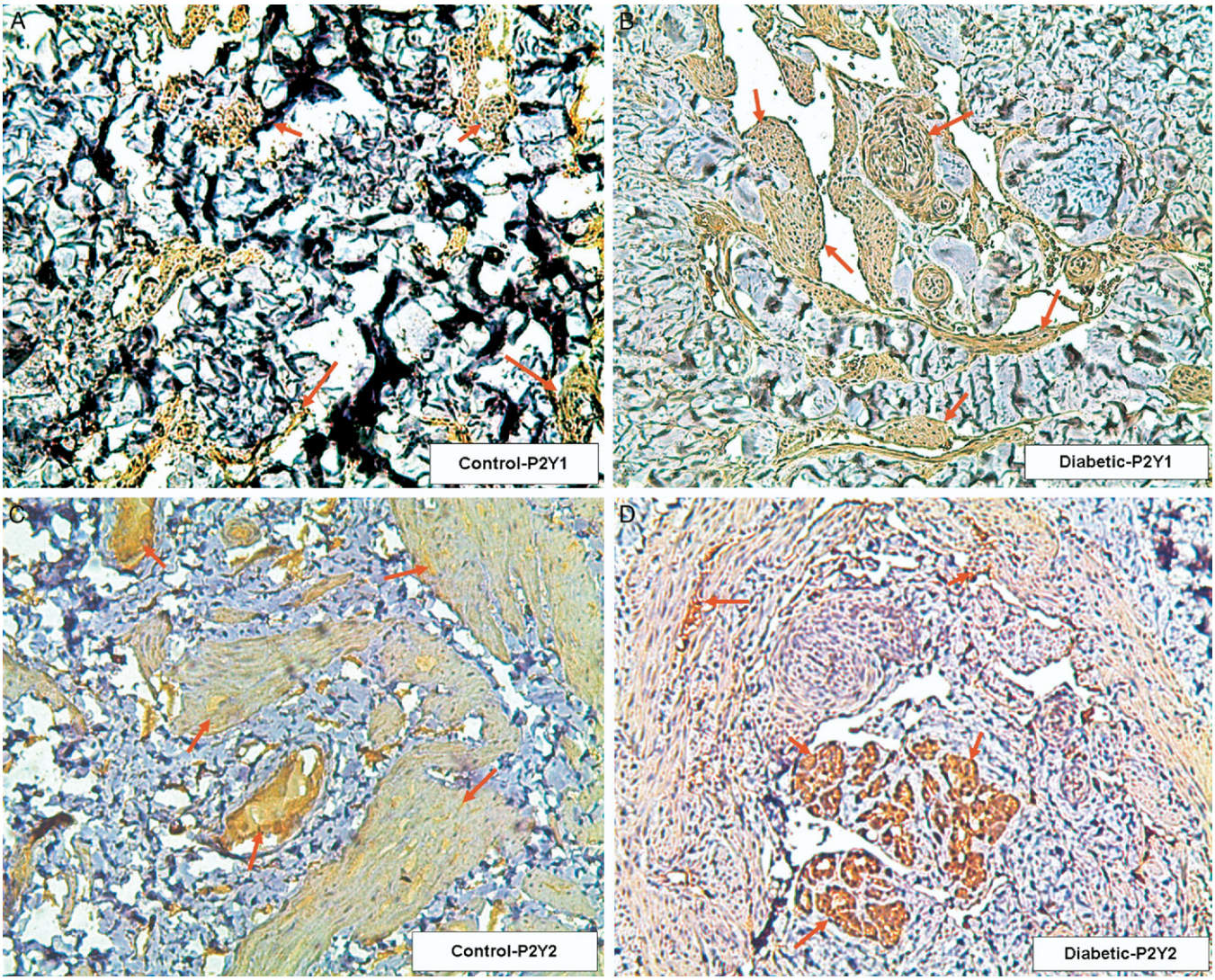

Figure 6. Representative immunohistochemical staining for P2Y1 receptors (arrows) in control (*A*) and diabetic (*B*) rat CCSM shows no positive immunostaining for P2Y1 in diabetic CCSM. Immunohistochemical staining for P2Y2 receptors in control (*C*) and diabetic (*D*) rat CCSM reveals abundant staining for P2Y2 receptors in diabetic CCSM. All photomicrographs were observed by 6 independent observers. Reduced from ×100.

tissue of diabetic rats. Lee et al observed immunoreactivity to the P2X1 receptor subtype in CCSM, which was responsible for contraction during the detumescence phase.[6] Additionally, the loss of the erectile response seen in the diabetic animal was most likely associated with maintaining vasoconstriction, at least partially mediated by PKC and/or RhoA/Rho-kinase signaling associated Ca2+ sensitization.[11] It is possible that enhanced P2X1 receptor activity caused by PKC or other unknown signaling pathways may contribute to increased contractile activity in diabetic ED cases.

The signal transduction mechanism is more complex for P2Y receptors than for P2X receptors. It is apparent that this pathway is relatively slower and, thus, the response time of P2Y receptor activation is longer than the rapid responses mediated by the ligand gated ion channels modulated by P2X receptors. According to our data the P2Y1 agonist 2-MeSATP alone in in vivo studies did not show significant improvement in the diabetic rat response. However, this agonist completely restored decreased relaxation in diabetic rat CCSM, which was clearly reversed by RB2. There appear to be contradictory data in the in vivo and in vitro responses to 2-MeSATP. We suggest that the restoration of erectile function in cases of diabetes is mediated by the effect of the P2Y1 receptor and 2-MeSATP may be one of the better P2Y1 agonists for drug targeting.

Furthermore, uracil nucleotide UTP, which is the preferred agonist at the P2Y2 receptor, is efficacious for potentiating the increase in ICP/MAP and pro-

longing detumescence time in diabetic animals. Results show that UTP was more potent than ATP for relaxing diabetic rat CCSM. These results were confirmed by immunohistochemical analysis with an antibody against the P2Y2 receptor subtype. This enhanced response may be due to degradation of UTP to uridine diphosphate and the fact that P2Y4 receptors may contribute to the enhanced relaxant effect of UTP. In support of this concept Lau et al found that UDP induced a 5% to 16% relaxation in the phenylephrine mediated CCSM response.[19] They suggested the presence of P2Y6 receptors in human cavernous tissue which, when activated, induced cavernous smooth muscle relaxation via nonneuronal and nonNO dependent mechanisms. Our data further revealed that functional P2Y1 purinoceptors are present on endothelial cells that line the lacunar spaces and P2Y2 purinoceptors are expressed intensely in CCSM. While P2Y1 receptors modulate endothelial function, P2Y2 receptors regulate CCSM activity. In addition, Obara et al noted that P2Y1 purinoceptors exist on endothelial cells that line the lacunar space.[7] Recently Calvert et al reported relaxation of CCSM via P2Y4 receptor activation, in addition to endothelium dependent relaxation via P2Y1 receptor activation.[20]

CONCLUSIONS

The current study confirms that ATP can contract CCSM by activating P2X receptors, while it evokes relaxation via P2Y1 and P2Y2 receptors in the diabetic rat. The net response of the penile tissues is a result of these 2 opposing effects of ATP at any given time. It is hypothesized that P2Y receptor mechanism(s) are dominant in the diabetic penis. Mechanisms regulating this receptor balance are as yet unclear and deserve further investigation. We suggest that attention be focused on P2Y receptor as a molecular target for the treatment of diabetic induced ED. It is our hypothesis that activating ATP based pathways can restore erectile function when NO bioavailability is impaired by diabetes.

ACKNOWLEDGMENTS

Drugs were obtained from Sigma-Aldrich®. Rabbit polyclonal antibodies were obtained from Santa-Cruz Biotechnology, Santa Cruz, California and Calbiochem®.

REFERENCES

1. Ralevic V and Burnstock G: Receptors for purines and pyrimidines. Pharmacol Rev 1998; **50:** 413.
2. Burnstock G and Knight GE: Cellular distribution and functions of P2 receptor subtypes in different systems. Int Rev Cytol 2004; **240:** 31.
3. Burnstock G: Purine and pyrimidine receptors. Cell Mol Life Sci 2007; **64:** 1471.
4. Tong YC, Broderick G, Hypolite J and Levin RM: Correlations of purinergic, cholinergic and adrenergic functions in rabbit corporal cavernosal tissue. Pharmacology 1992; **45:** 241.
5. Takahashi Y, Ishii N, Lue TF and Tanagho EA: Effects of adenosine on canine penile erection. J Urol 1992; **148:** 1323.
6. Lee HY, Bardini M and Burnstock G: P2X receptor immunoreactivity in the male genital organs of the rat. Cell Tissue Res 2000; **300:** 321.
7. Obara K, Lepor H and Walden PD: Localization of P2Y1 purinoceptor transcripts in the rat penis and urinary bladder. J Urol 1998; **160:** 587.
8. Gur S, Kadowitz PJ and Hellstrom WJ: Purinergic (P2) receptor control of lower genitourinary tract function and new avenues for drug action: an overview. Curr Pharm Des 2007; **13:** 3236.
9. Saenz de Tejada I, Goldstein I, Azadzoi K, Krane RJ and Cohen RA: Impaired neurogenic and endothelium-mediated relaxation of penile smooth muscle from diabetic men with impotence. N Engl J Med 1989; **320:** 1025.
10. Rehman J, Chenven E, Brink P, Peterson B, Walcott B, Wen YP et al: Diminished neurogenic but not pharmacological erections in the 2- to 3-month experimentally diabetic F-344 rat. Am J Physiol 1997; **272:** H1960.
11. Bivalacqua TJ, Kendirci M, Champion HC, Hellstrom WJ, Andersson KE and Hedlund P: Dysregulation of cGMP-dependent protein kinase 1 (PKG-1) impairs erectile function in diabetic rats: influence of in vivo gene therapy of PKG1alpha. BJU Int 2007; **99:** 1488.
12. Noto T, Inoue H, Mochida H and Kikkawa K: Role of adenosine and P2 receptors in the penile tumescence in anesthetized dogs. Eur J Pharmacol 2001; **425:** 51.
13. Rapaport E: Experimental cancer therapy in mice by adenine nucleotides. Eur J Cancer Clin Oncol 1988; **24:** 1491.
14. Shabbir M, Thompson C, Jarmulowiczc M, Mikhailidis D and Burnstock G: Effect of extracellular ATP on the growth of hormone-refractory prostate cancer in vivo. BJU Int 2008; **102:** 108.
15. Agteresch HJ, Burgers SA, van der Gaast A, Wilson JH and Dagnelie PC: Randomized clinical trial of adenosine 5′-triphosphate on tumor growth and survival in advanced lung cancer patients. Anticancer Drugs 2003; **14:** 639.
16. Dunn PM, Zhong Y and Burnstock G: P2X receptors in peripheral neurons. Prog Neurobiol 2001; **65:** 107.
17. Angulo J, Cuevas P, Fernández A, Allona A, Moncada I, Martín-Morales A et al: Enhanced thromboxane receptor-mediated responses and impaired endothelium-dependent relaxation in human corpus cavernosum from diabetic impotent men: role of protein kinase C activity. J Pharmacol Exp Ther 2006; **319:** 783.
18. Tien XY, Brasitus TA, Kaetzel MA, Dedman JR and Nelson DJ: Activation of the cystic fibrosis transmembrane conductance regulator by cGMP in the human colonic cancer cell line, Caco-2. J Biol Chem 1994; **269:** 51.
19. Lau DH, Metcalfe MJ, Mumtaz FH, Mikhailidis DP and Thompson CS: Purinergic modulation of human corpus cavernosum relaxation. Int J Androl 2007; Epub ahead of print.
20. Calvert RC, Khan MA, Thompson CS, Mikhailidis DP and Burnstock G: A functional study of purinergic signalling in the normal and pathological rabbit corpus cavernosum. BJU Int 2008; **101:** 1043.