ELSEVIER

European Journal of Pharmacology 349 (1998) 75–82

ejp

Evidence for purinergic neurotransmission in the urinary bladder of pithed rats

Sharath S. Hegde *, Dan A. Mandel, Mary R. Wilford, Stephanie Briaud, Anthony P.D.W. Ford, Richard M. Eglen

Center for Biological Research, Neurobiology Unit, Roche Bioscience, 3401 Hillview Avenue, Palo Alto, CA 94304, USA

Received 1 December 1997; revised 12 February 1998; accepted 24 February 1998

Abstract

The purpose of this study was to investigate the contribution of adenosine-5′-triphosphate (ATP) to segmental (L6-S2) spinal electrical stimulation evoked increases in intra-vesical pressure in pithed rats. Exogenous ATP and substance P produced dose-dependent increases in intra-vesical pressure ($ED_{10\ mmHg}$ (dose required to elicit 10 mmHg increase in intra-vesical pressure) = 1.7 mg/kg and 1.1 μg/kg, i.v., respectively). Desensitisation (or antagonism) of P_{2x} purinoceptors with α,β-methylene ATP (α,β-meATP; 30 μg/kg per min, i.v.) or pyridoxalphosphate-6-azophenyl-2′,4′-disulfonic acid (PPADS; 10 mg/kg, i.v.) significantly ($p < 0.05$) antagonized the intra-vesical pressure responses to ATP (> 8 and 3.6-fold increase in $ED_{10\ mmHg}$, respectively) but had no significant effect on intra-vesical pressure responses to substance P. Spinal stimulation evoked frequency-dependent increases in intra-vesical pressure ($EF_{20\ mmHg}$ (frequency required to produce 20 mmHg increase in intra-vesical pressure) = 3.4 Hz). Blockade of muscarinic cholinoceptors and adrenoceptors with atropine (3 mg/kg, i.v.), propranolol (3 mg/kg, i.v.) and phentolamine (10 mg/kg, i.v.) produced marginal attenuation of the intra-vesical pressure responses to spinal stimulation indicating a major non-adrenergic non-cholinergic (NANC) component in the overall response. The NANC responses were significantly ($p < 0.05$) antagonized by α,β-meATP (30 μg/kg per min, i.v.) and PPADS (10 mg/kg, i.v.) (> 2.6-fold increase in $EF_{20\ mmHg}$), consistent with involvement of a purinergic neurotransmitter, presumably ATP. Comparative studies in young (4–6 months) and old (21–23 months) Fischer rats revealed no age-dependent changes in the relative contribution of the cholinergic and purinergic systems, with the latter being the dominant one. These findings suggest that purinergic neurotransmission, presumably mediated by ATP acting via P_{2x} purinoceptors, represents a major component of excitatory innervation to the urinary bladder in pithed rats.

Keywords: Purinoceptor; Purinergic; Bladder; PPADS (pyridoxalphosphate-6-azophenyl-2′,4′-disulfonic acid); α,β-methylene ATP

1. Introduction

Parasympathetic nerves, originating from the sacral and lower lumbar regions of the spinal cord, represent the principal excitatory innervation to the urinary bladder (see De Groat et al., 1993; Hoyle and Burnstock, 1993 for reviews). The contribution of acetylcholine to parasympathetically-evoked bladder contractions, in vivo and in vitro, is indisputable. In several species, the additional involvement of a non-adrenergic, non-cholinergic (NANC) transmitter has been proposed, although its relative contribution is dependent on the species under study (see Hoyle, 1995, for review). Rats, in particular, have a dominant NANC innervation to the urinary bladder (Carpenter, 1977; Tong et al., 1996).

Pharmacological studies, using rat isolated bladder strips, have shown that the NANC component is mimicked by exogenous adenosine-5′-triphosphate (ATP) and desensitized by α,β-methylene ATP (α,β meATP) implying a role of endogenous ATP (Luheshi and Zar, 1990a; Parija et al., 1991; Maggi, 1991; Tong et al., 1997). However, purinergic excitatory transmission in rats has seldom been definitively demonstrated in vivo. Igawa et al. (1993) showed that P_{2x} receptor desensitization with α,β-meATP, decreased micturition pressure and increased bladder capacity and residual volume in conscious rats, suggesting a role of ATP in the micturition reflex. This study should be interpreted with caution since the specificity of α,β-meATP in vivo, which has been questioned (Dalziel et al.,

* Corresponding author. Tel.: +1-650-852-3198; fax: +1-650-354-7400; e-mail: sharath.hegde@roche.com

0014-2999/98/$19.00

1990), was not evaluated. Furthermore, the site of involvement of ATP (i.e., supra-spinal, spinal or peripheral) could not be ascertained.

The objective of this study was, therefore, to investigate more directly the role of peripheral purinergic transmission to the rat bladder in vivo. For this purpose, we employed the pithed rat model to investigate the contribution of ATP to segmental (L6-S2) spinal electrical stimulation evoked increases in intra-vesical pressure in pithed Sprague–Dawley rats using α,β-meATP and also pyridoxalphosphate-6-azophenyl-2′-4′-disulfonic acid (PPADS), a selective P_{2x}-receptor antagonist (Zignshin et al., 1993) as pharmacological tools. Additionally, since the incidence of lower urinary tract disorders is higher in the elderly (Resnick, 1995), a secondary goal of the study was to assess the relative contribution of cholinergic and NANC transmission in young and aged Fischer rats.

2. Methods

2.1. General

Female Sprague–Dawley rats (3–6 months of age; 150–350 g) and female Fischer rats (4–6 months (young) and 21–23 months (old) of age; 150–350 g) were obtained from Charles River Laboratories (Hollister, CA, USA). On the day of the study, animals were weighed, anesthetized with pentobarbital (60 mg/kg, i.p.), and traecheotomised (PE-240 tubing). One femoral artery and vein were catheterized (PE-50 tubing) for measurement of blood pressure and administration of drugs, respectively. The pelvic viscera were exposed, and the two ureters were ligated and cut proximal to the ligation in order to allow urine to drain into cotton wads. The urinary bladder was cannulated with PE-50 tubing via the urethra, and the cannula was tied around the external urethral orifice. The bladder cannula was connected to a three-way connector to allow measurement of intra-vesical pressure and injection of saline. After completion of surgery, rats were pithed via the orbit of the eye. The stainless steel pithing rod was insulated with Teflon, except for the 1 cm tip which served as the electrode. The electrode was positioned 14.4 cm from the orbit of the eye, in the (L6-S2) region of the spinal cord (Gillespie et al., 1970). A second indifferent electrode was placed subcutaneously on the ventral side. Rats were artificially ventilated at 60 cycles/min with a volume of 1.5 ml/100 g of body weight, and body temperature was maintained at 37°C by placing the animals on a heating pad. The bladder was filled with 0.5 ml saline. Animals were allowed to stabilize for 20 min before the start of the experiment. In some experiments (experimental protocols I, IV and V), the pithing rod was stimulated electrically (Grass Instruments, Models S48 and S48F) to excite pre-ganglionic parasympathetic nerves which traverse to pelvic organs including the urinary bladder and changes in intra-vesical pressure were recorded. In other experiments (experimental protocols II and III), the effects of intravenously administered agonists on diastolic blood pressure and intra-vesical pressure were studied. All animals were pre-treated with tubocurarine (1 mg/kg, i.v.) to block neuromuscular transmission in skeletal muscles.

Five separate experimental protocols were adopted, as can be seen in the following.

2.2. Experimental protocol I

The purpose of these experiments was to evaluate the relative contributions of acetylcholine and NANC neurotransmitter(s) to spinal stimulation-evoked bladder contractions. The pithing rod was stimulated at varying frequencies (1, 2, 4, 8 and 16 Hz, 80 V, 0.1 ms pulse width for 10 s) to generate a frequency-response (change in intra-vesical pressure) curve. Subsequently, the animals were treated with either atropine (3.16 mg/kg, i.v.) alone or atropine (3.16 mg/kg) plus propranolol (3.16 mg/kg, i.v.) plus phentolamine (10 mg/kg, i.v.) or chlorisondamine (5 mg/kg, i.v.) alone. Fifteen minutes later, a second frequency-response curve was constructed.

2.3. Experimental protocol II

The intent of these experiments was to determine the effects of α,β-meATP (P_{2x}-receptor desensitization) and PPADS (P_{2x} receptor antagonism) on the responses to exogenous ATP. In addition to atropine (3.16 mg/kg, i.v.), animals were also pre-treated with propranolol (3.16 mg/kg) in these experiments to eliminate β-adrenoceptor-mediated relaxation of the bladder (Maggi and Meli, 1982) by circulating catecholamines. A non-cumulative ATP dose-response curve (0.1–10.0 mg/kg, i.v.) was performed. Subsequently, either α,β-meATP (infused at 30 μg/kg/min, i.v.) or PPADS (10 mg/kg, bolus, i.v.) was administered. Thereafter (i.e., 30 min after the initiation of the α,β-meATP infusion or 5 min after administration of PPADS), a second non-cumulative ATP dose-response curve was constructed.

2.4. Experimental protocol III

These experiments were performed to determine the specificity of α,β-meATP and PPADS by evaluating their effects on the bladder responses to substance P. Preliminary experiments showed that two reproducible dose-response curves could not be obtained in the same animal. Therefore, a single curve to substance P was constructed per animal and the responses were expressed as a percent of the response to ATP in the same animal. Animals were pre-treated with atropine (3.16 mg/kg, i.v.) and propranolol (3.16 mg/kg) in these experiments. After obtaining a control response to ATP (3 mg/kg, i.v.), the animals were treated with either α,β-meATP (infused at 30 μg/kg/min, i.v.) or PPADS (10 mg/kg, i.v.). Thereafter, a non-cumulative substance P dose-response curve (0.316–31.6 μg/kg, i.v.) was performed.

2.5. Experimental protocol IV

The goal of these experiments was to determine the effects of α,β-meATP and PPADS on NANC bladder responses to parasympathetic nerve stimulation. Animals were pre-treated with atropine (3.16 mg/kg, i.v.) and propranolol (3.16 mg/kg) in these experiments. A frequency-response curve (1, 2, 4, 8 and 16 Hz, 80 V, 0.1 ms pulse width for 10 s) was performed. Subsequently, either α,β-meATP (infused at 30 μg/kg/min, i.v.) or PPADS (10 mg/kg, bolus, i.v.). Thereafter, a second frequency-response curve was constructed.

2.6. Experimental protocol V

The objective of these experiments was to determine the relative contribution of cholinergic and purinergic neurotransmission towards parasympathetically-evoked contractions in young (4–6 months) and old (21–23 months) Fischer rats. A frequency-response curve (1, 2, 4, 8 and 16 Hz, 80 V, 0.1 ms pulse width, for 10 s) was performed. After 10 min, atropine (3.16 mg/kg, i.v.) was administered and 15 min thereafter a second frequency-response curve was performed. Subsequently, PPADS (10 mg/kg, i.v.) was administered and 40 min later, a third frequency-response curve was generated. Ten minutes later, chlorisondamine (5.0 mg/kg, i.v.) was administered and 15 min thereafter, a fourth frequency-response curve was obtained.

2.7. Data analysis

All data are expressed as mean $\pm$ standard error of mean (S.E.M.) or with 95% confidence intervals in parentheses. Estimates of $ED_{10\ mmHg}$ (dose of agonist required to elicit 10 mmHg increase in intra-vesical pressure) and $EF_{20\ mmHg}$ (frequency of spinal stimulation required to produce 20 mmHg increase intra-vesical pressure) were calculated using Seemingly Unrelated Non-linear Regression (SUNR) analysis (Leung et al., 1992).

2.8. Drugs

α,β-Methylene ATP, atropine, propranolol, substance P, and ATP were obtained from Sigma Chemical (MO, USA). Phentolamine, PPADS and tubocurarine were obtained from Research Biochemicals (MA, USA). Chlorisondamine was synthesized at Roche Bioscience (Palo Alto, CA, USA).

3. Results

3.1. Baseline variables

The baseline intra-vesical pressure and diastolic blood pressure in the animals were 7.3 ± 0.4 and 34.6 ± 0.7 mmHg ($n = 104$), respectively. Administration of atropine (3 mg/kg, i.v.), phentolamine (10 mg/kg, i.v.), propranolol (3 mg/kg, i.v.), chlorisondamine (5 mg/kg, i.v.) and PPADS (10 mg/kg, i.v.) had no significant effect on intra-vesical pressure or diastolic blood pressure. Infusion of α,β-meATP (30 μg/kg/min, i.v.) produced an increase in intra-vesical pressure (8.8 ± 1.9 mmHg) and diastolic blood pressure (57.1 ± 5.3 mmHg); these responses were transient and returned back to baseline within approximately 5 min.

3.2. Effects of muscarinic cholinoceptor blockade, adrenoceptor blockade, and ganglionic nicotinic receptor blockade on spinal stimulation-evoked increases in urinary bladder pressure (Experimental protocol I)

Spinal stimulation had no significant effect on diastolic blood pressure but produced frequency-dependent increases in intra-vesical pressure (Fig. 1A and B). The

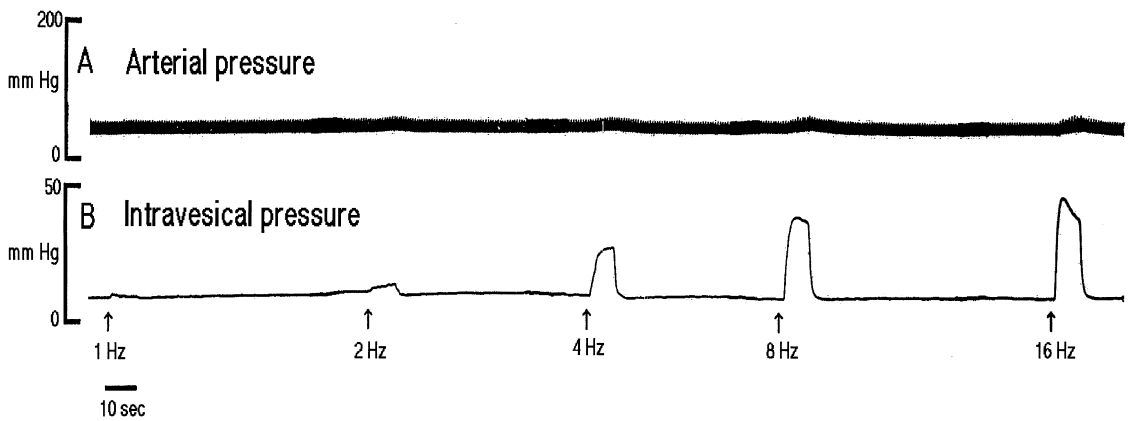

Fig. 1. Polygraph tracing showing the effects of segmental spinal stimulation on arterial pressure (A) and intra-vesical pressure (B) in pithed rats.

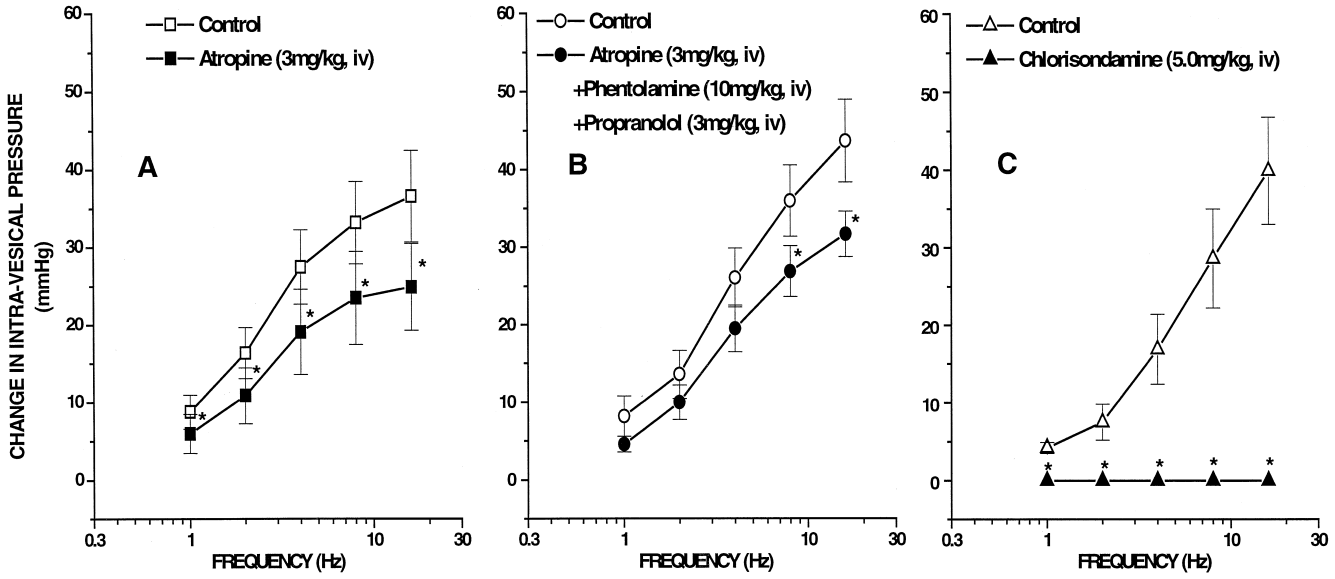

Fig. 2. Effects of atropine (A), atropine plus phentolamine plus propranolol (B), and chlorisondamine (C) on segmental spinal stimulation-evoked increases in intra-vesical pressure in pithed rats. $n = 4–7$ per group. $* p < 0.05$ vs. control.

estimated $EF_{20\ mmHg}$ was 3.4 (2.1–5.3) Hz and the maximum increase in intra-vesical pressure, at the frequencies studied, was 40.3 (33.7–46.9) mmHg ($n = 17$). Antagonism of muscarinic receptors with atropine (3 mg/kg, i.v.) produced a significant, rightward shift of the frequency-response curve ($\sim$ 2-fold increase in $EF_{20\ mmHg}$) (Fig. 2A). Additional blockade of α and β-adrenoceptors, with phentolamine (10 mg/kg, i.v.) plus propranolol (3 mg/kg, i.v.), produced no further shift of the frequency-response curve (Fig. 2B). Treatment with chlorisondamine (5 mg/kg, i.v.), a ganglion blocker, completely abolished spinal stimulation-evoked increase in intra-vesical pressure (Fig. 2C).

3.3. Effects of PPADS and α,β-meATP on ATP-induced increases in intra-vesical pressure and diastolic blood pressure (Experimental protocol II)

Intravenously administered ATP produced dose-dependent increases in intra-vesical pressure. The estimated $ED_{10\ mmHg}$ was 1.7 (1.0–2.7) mg/kg, i.v. and the maximum increase in intra-vesical pressure, at the doses studied, was 16.5 (14.0–19.0) mmHg ($n = 27$). Desensitization

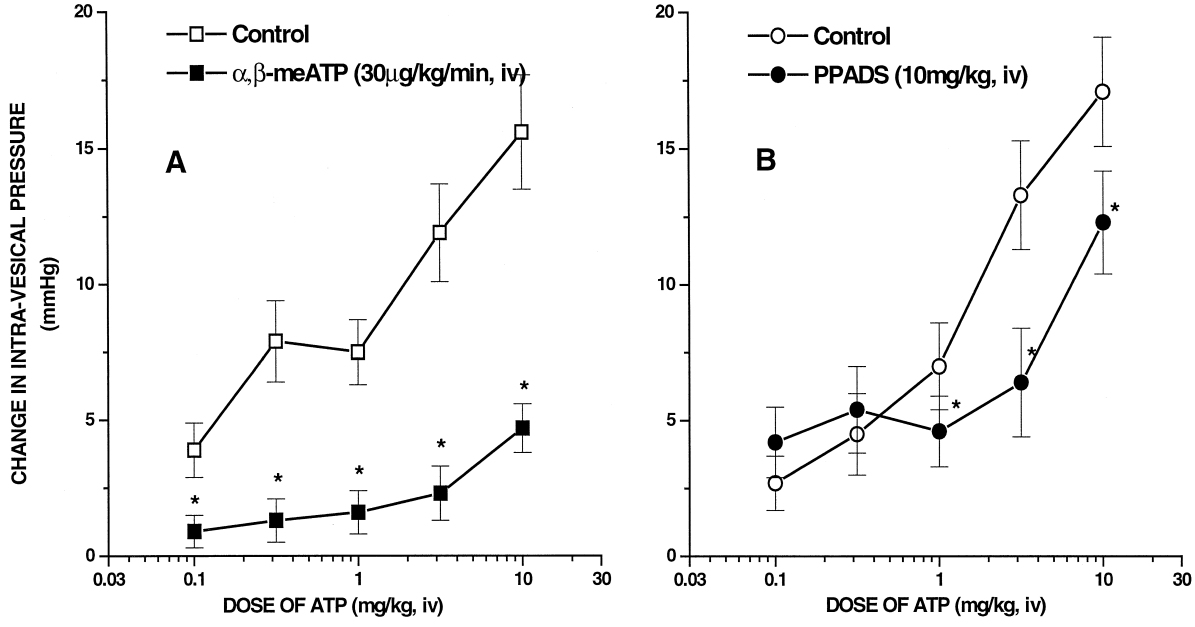

Fig. 3. Effects of α,β-meATP (A) and PPADS (B) on ATP induced increases in intra-vesical pressure in pithed rats. $n = 7–8$ per group. $* p < 0.05$ vs. control.

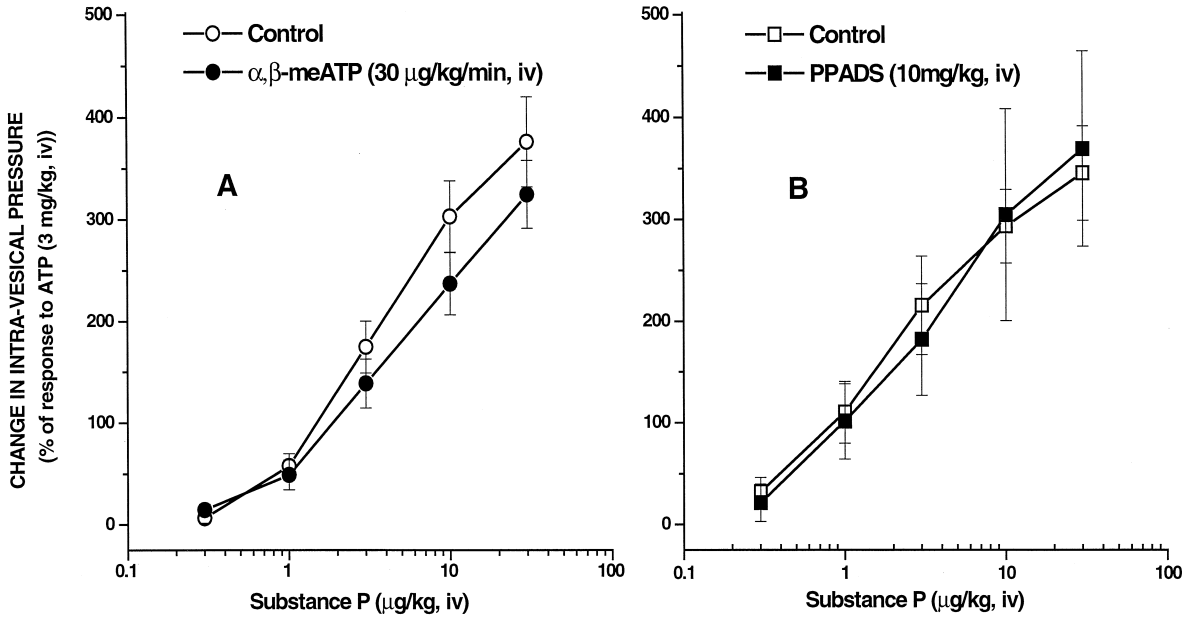

Fig. 4. Effects of α,β-meATP (A) and PPADS (B) on substance P induced increases in intra-vesical pressure in pithed rats. $n = 5–7$ per group.

of P_{2x} purinoceptors with α,β-meATP (30 μg/kg/min, i.v.) or blockade of P_{2x} purinoceptors with PPADS (10 mg/kg, i.v.) antagonized the intra-vesical pressure responses to ATP (approximately >8- and 3.6-fold increases in $ED_{10\ mmHg}$, respectively (Fig. 3A and B).

3.4. Effects of PPADS and α,β-meATP on substance P-induced increases in urinary bladder pressure (Experimental protocol III)

Substance P produced dose-dependent increases in intra-vesical pressure. The estimated $ED_{10\ mmHg}$ was 1.1 (0.7–1.9) μg/kg, i.v. and the maximum increase in intra-vesical pressure, at the doses studied, was 39.7 (31.0–48.3) mmHg ($n = 12$). Neither α,β-meATP (30 μg/kg/min, i.v.) nor PPADS (10 mg/kg, i.v.) had any significant effect on substance P-induced increases in intra-vesical pressure (Fig. 4A and B)

3.5. Effects of PPADS and α,β-meATP on spinal stimulation-evoked, atropine-resistant (NANC), increases in intra-vesical pressure (Experimental protocol IV)

In atropine-pretreated rats, spinal stimulation produced frequency-dependent increases in urinary bladder pressure.

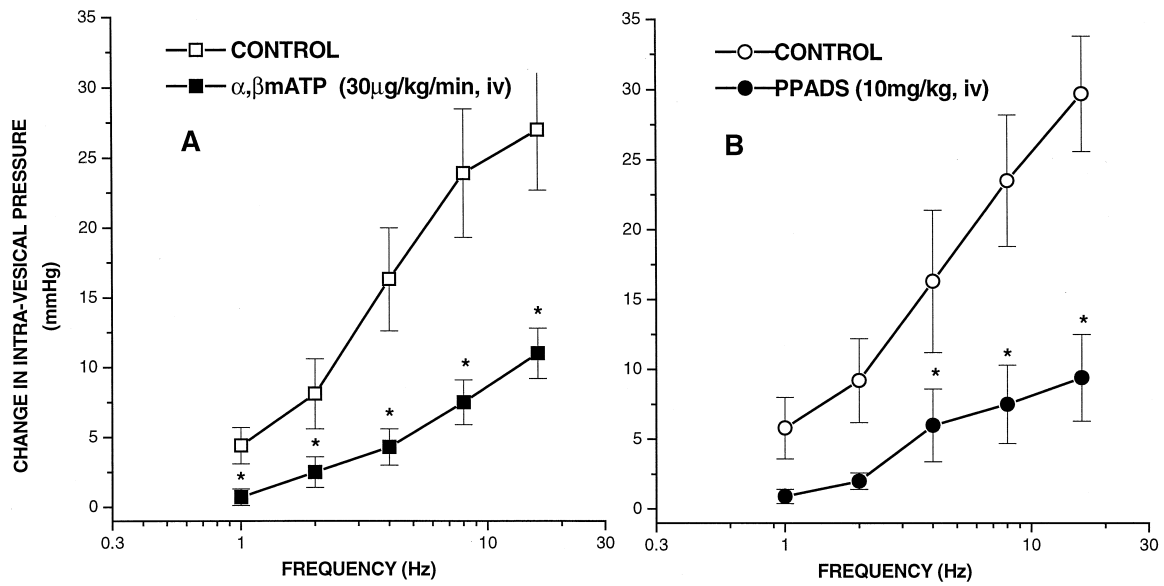

Fig. 5. Effects of α,β-meATP (A) and PPADS (B) on segmental spinal stimulation evoked (non-adrenergic non-cholinergic component) increases in intra-vesical pressure in pithed rats. $n = 5$ per group. $* p < 0.05$ vs. control.

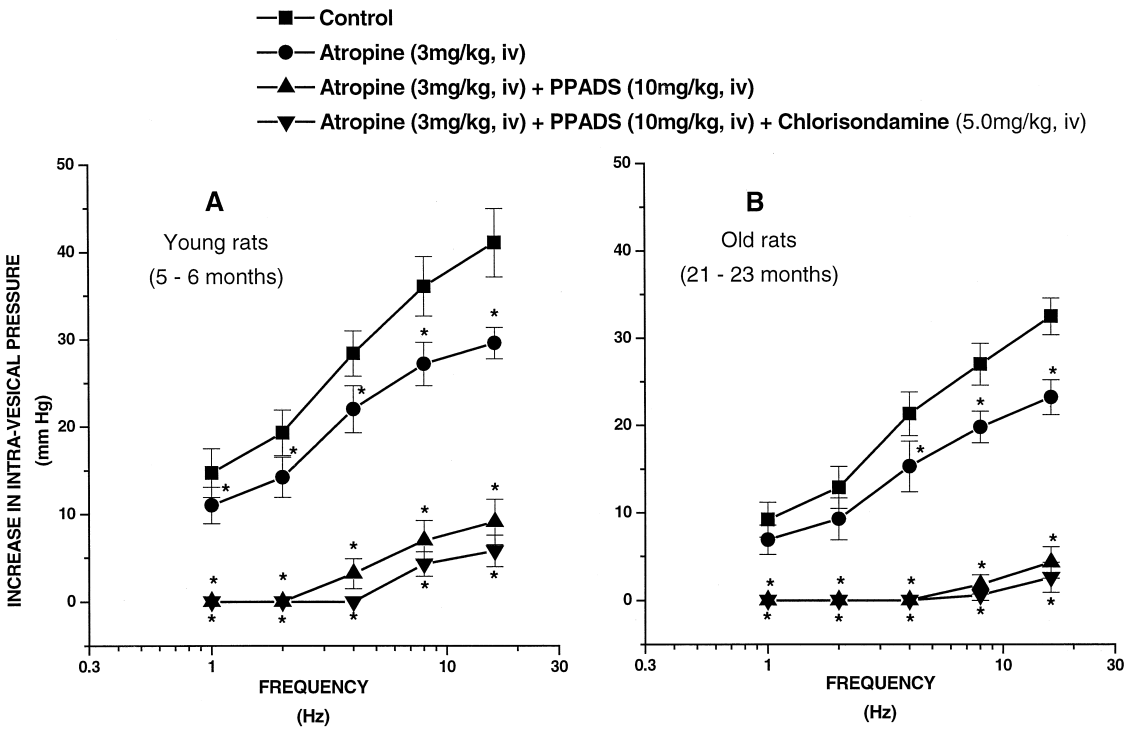

Fig. 6. Effects of atropine, PPADS and chlorisondamine on segmental spinal stimulation evoked increases in intra-vesical pressure in young (A) and old (B) Fischer rats. $n = 5$ per group. $* p < 0.05$ vs. control.

α,β-meATP (30 μg/kg/min, i.v.) and PPADS (10 mg/kg, i.v.) significantly attenuated the NANC bladder responses to spinal stimulation (approximately $>$ 2.6-fold increases in $EF_{20\ mmHg}$) (Fig. 5A and B).

3.6. Effect of age on the excitatory transmission in the urinary bladder of Fischer rats (Experimental protocol V).

Spinal stimulation evoked frequency-dependent increases in intra-vesical pressure in both young and old Fischer rats (Fig. 6A and B). The $EF_{20\ mmHg}$ estimates were significantly different from each other (1.9 (1.5–2.4) and 3.8 (3.4–4.3) in young and aged animals, respectively). At the frequencies tested, the maximum increase in intra-vesical pressure was significantly greater in young (41.1 $\pm$ 3.9 mmHg) as compared to old (32.5 $\pm$ 2.1 mmHg) rats. Atropine and PPADS produced comparable inhibition of the cholinergic and non-cholinergic components, respectively, in both young and old rats (Fig. 6A and B).

4. Discussion

The process of micturition is critically dependent on the operation of a spinal–bulbospinal reflex which comprises afferent (sensory) and efferent (motor) peripheral pathways which are integrated and co-ordinated at spinal and supraspinal centers (De Groat et al., 1993). The lumbosacral (L6-S2) parasympathetic outflow provides the excitatory motor input to the urinary bladder of rats. In vitro pharmacological studies in the rat bladder have shown that excitatory (contractile) neurotransmission is mediated by cholinergic (i.e., via acetylcholine) and purinergic (i.e., via ATP) pathways (Hoyle and Burnstock, 1993; Hoyle, 1995). However, unambiguous evidence for peripheral cholinergic/purinergic co-transmission in the urinary bladder of rats in vivo has seldom been obtained owing to the complexity of the micturition reflex in the whole animal and the lack of reliable purinoceptor-specific pharmacological tools for use in vivo. The pithed rat model, employed in the present study, is useful in that it allows one to study the autonomic innervation to peripheral tissues in isolation without the confounding influence of central pathways. Using this model, we have shown that purinergic transmission mediates a sizeable component of parasympathetically-evoked contractions.

Spinal stimulation evoked reproducible and frequency-dependent increases in bladder pressure without any changes in arterial pressure, consistent with selective stimulation of the lumbosacral parasympathetic outflow. The sensitivity of the bladder responses to chlorisondamine suggests that the responses are mediated via a neurogenic mechanism (i.e., via autonomic nerves). Treatment with atropine produced only marginal attenuation of the neurogenic bladder responses indicating a minor cholinergic contribution to the overall response. The involvement of the adrenergic system can be discounted inasmuch as phentolamine plus propranolol produced no further inhibi-

tion of bladder contractions. Collectively, these data suggest that excitatory innervation to the urinary bladder of rats is under dominant non-adrenergic non-cholinergic (NANC) control.

We next sought to determine whether the NANC component of bladder contraction is purinergically-mediated using α,β-meATP and PPADS as pharmacological tools. Desensitization of P_{2x} purinoceptors by α,β-meATP and/or antagonism of P_{2x} purinoceptors by PPADS has been commonly used to uncover purinoceptor mediated responses in pharmacological studies performed in vitro (Abbrachio and Burnstock, 1994). However, owing to a paucity of data on the usefulness of these drugs in an in vivo setting, it was pertinent to evaluate the pharmacological specificity of these drugs in the pithed rat. For this purpose, we examined the influence of α,β-meATP and PPADS on the responses to exogenous ATP and substance P. Both of these agonists produced dose-dependent increases in bladder pressure, although the responses to substance P, besides being more long-lasting, also had a higher maximum. Both α,β-meATP (30 μg/kg/min, i.v.) and PPADS (10 mg/kg, i.v.) antagonised the bladder responses to ATP but not substance P. It is intriguing that PPADS, whose affinity for P_{2x} receptors is in the low μM range, was reasonably potent in antagonizing the responses to ATP. It should be noted, however, that PPADS does not interact with P_{2x} purinoceptors in a strictly reversible and competitive manner in vitro (Evans et al., 1995). Nevertheless, the data suggest that, at the doses studied, both α,β-meATP and PPADS selectively desensitize (or antagonize) purinoceptors (presumably P_{2x}) and therefore lends support to their utility in elucidating the role of P_{2x} purinoceptors in mediating excitatory transmission in the urinary bladder in vivo. Indeed, subsequent experiments showed that the NANC bladder responses were markedly suppressed, but not abolished, by α,β-meATP and PPADS, consistent with the involvement of a purinergic transmitter, presumably ATP. Incomplete desensitization (or antagonism) of P_{2x} receptors or the existence of non-P_{2x} purinoceptors in the rat bladder (Hashimoto and Kokubun, 1995) could account for the residual contractile component although the involvement of other NANC transmitter(s) cannot be dismissed.

It is well known that urinary incontinence is more prevalent in the elderly, affecting 5–15% of the elderly living at home and over 50% of the institutionalized population (Resnick, 1995). Incontinence in the elderly is associated with increased incidence of uninhibited detrusor contractions and decreased bladder capacity. Studies in animals have shown age-dependent changes in pharmacological responsiveness of the bladder. For example, sensitivity of the bladder base to muscarinic agonists is greater in aged rats compared to younger controls (Ordway et al., 1986). It was therefore of interest to determine whether the relative contribution of the cholinergic and purinergic pathways to excitatory transmission in the bladder is altered with age, particularly since one study has reported age-dependent augmentation of the contractile responsiveness of the rat bladder to exogenous ATP (Saito et al., 1991). The results obtained in young and old Fischer rats suggest that although the cholinergic and purinergic systems participate in neurogenically-mediated bladder contractions in both groups, their relative contribution appears to be very similar. These data, however, do not rule out the possibility of early (i.e., <4 months) developmental changes in the relative predominance of the two systems.

In conclusion, the findings of this study suggest that purinergic neurotransmission, presumably mediated by ATP acting through P_{2x} purinoceptors, represents a major component of excitatory innervation to the urinary bladder of young and aged pithed rats. Controversy has existed regarding the involvement of NANC transmitter(s) in neurogenic contractions of the human bladder. Although some studies have failed to show the existence of any meaningful atropine-resistant component in the human bladder (Sibley, 1984; Palfrey et al., 1984; Kinder and Mundy, 1985; Chen et al., 1994), other investigations have reported a sizeable purinergic component whose contribution is enhanced in diseased states such as interstitial cystitis and detrusor instability (Husted et al., 1983; Sjogren et al., 1982; Hoyle et al., 1989; Luheshi and Zar, 1990b; Palea et al., 1993). If these latter findings are confirmed, it is likely that development of selective P_{2x} receptor antagonists may be therapeutically rewarding.

References

Abbrachio, M.P., Burnstock, G., 1994. Purinoceptors: are there families of P_{2x} and P_{2y} purinoceptors?. Pharmacol. Ther. 64, 445–475.

Carpenter, F.G., 1977. Atropine resistance and muscarinic receptors in the rat urinary bladder. Br. J. Pharmacol. 59, 43–49.

Chen, T.F., Doyle, P.T., Ferguson, D.R., 1994. Inhibition in the human urinary bladder by gamma-amino-butyric acid. Br. J. Urol. 73, 250–255.

Dalziel, H.H., Gray, G.A., Drummond, R.M., Furman, B.L., Sneddon, P., 1990. Investigation of the selectivity of α,β-methylene ATP in inhibiting vascular responses of the rat in vivo and in vitro. Br. J. Pharmacol. 99, 820–824.

De Groat, W.C., Booth, A.M., Yoshimura, N., 1993. Neurophysiology of micturition and its modification in animal models of human disease. In: Maggi, C.A. (Ed.), Nervous Control of the Urogenital System. Harwood Academic Publishers, pp. 227–290.

Evans, R.J., Lewis, C., Buell, G., Valera, S., North, R.A., Surprenant, A., 1995. Pharmacological characterization of heterologously expressed ATP-gated cation channels (P_{2x} purinoceptors). Mol. Pharmacol. 48, 178–183.

Gillespie, J.S., Maclaren, A., Pollock, D., 1970. A method of stimulating different segments of the autonomic outflow from the spinal column to various organs in the pithed cat and rat. Br. J. Pharmacol. 40, 257–267.

Hashimoto, M., Kokubun, S., 1995. Contribution of P2-purinoceptors to neurogenic contraction of rat urinary bladder smooth muscle. Br. J. Pharmacol. 115, 636–640.

Hoyle, C.H.V., 1995. Non-adrenergic, non-cholinergic control of the urinary bladder. World J. Urol. 12, 233–244.

Hoyle, C.H.V., Burnstock, G., 1993. Postganglionic efferent transmission in the bladder and urethra. In: Maggi, C.A. (Ed.), Nervous Control of the Urogenital Tract. Harwood Academic Publishers, pp. 349–381.

Hoyle, C.H.V., Chapple, C., Burnstock, G., 1989. Isolated human bladder: evidence for an adenine dinucleotide acting on P_{2x}-purinoceptors and for purinergic transmission. Eur. J. Pharmacol. 174, 115–118.

Husted, S., Sjogren, C., Andersson, K.E., 1983. Direct effects of adenosine nucleotides on isolated human bladder and their influence on electrically-induced contractions. J. Urol. 130, 392–398.

Igawa, Y., Mattiasson, A., Andersson, K.-E., 1993. Functional importance of cholinergic and purinergic neuro-transmission for micturition contraction in the normal, unanesthetized rat. Br. J. Pharmacol. 109, 473–479.

Kinder, R.B., Mundy, A.R., 1985. Atropine blockade of nerve-mediated stimulation of the human detrusor. Br. J. Urol. 57, 418–421.

Leung, E., Michelson, S., Villarubia, C., Perkins, L.A., Eglen, R.M., 1992. Analysis of concentration-response relationships by seemingly-unrelated non-linear regression (SUNR) technique. J. Pharmacol. Methods 4, 1209–1216.

Luheshi, G., Zar, A., 1990a. Purinoceptor desensitization impairs but does not abolish the non-cholinergic motor transmission in rat isolated urinary bladder. Eur. J. Pharmacol. 185, 203–208.

Luheshi, G.N., Zar, M.A., 1990b. Presence of non-cholinergic motor transmission in human isolated bladder. J. Pharm. Pharmacol. 42, 223–224.

Maggi, C.A., 1991. Omega conotoxin and pre-junctional modulation of the biphasic response of the rat isolated urinary bladder to single pulse electrical field stimulation. J. Auton. Pharmacol. 11, 295–304.

Maggi, C.A., Meli, A., 1982. Modulation by beta adrenoceptors of spontaneous contractions of the rat urinary bladder. J. Auton. Pharmacol. 2, 255–260.

Ordway, G.A., Esbenshade, T.A., Kolta, M.G., Gerald, M.C., Wallace, L.J., 1986. Effect of age on cholinergic muscarinic responsiveness and receptors in the rat urinary bladder. J. Urol. 136, 492–496.

Palea, S., Artibani, W., Ostardo, E., Trist, D.G., Pietra, C., 1993. Evidence for purinergic neurotransmission in human urinary bladder affected by interstitial cystitis. J. Urol. 150, 2007–2012.

Palfrey, E.L.H., Fry, C.H., Shuttleworth, K.E.D., 1984. A new in vitro microperfusion technique for investigation of human detrusor muscle. Br. J. Urol. 56, 635–640.

Parija, S.C., Raviprakash, V., Mishra, S.K., 1991. Adenosine- and α,β-methylene ATP-induced differential inhibition of cholinergic and non-cholinergic neurogenic responses in rat urinary bladder. Br. J. Pharmacol. 102, 396–400.

Resnick, N.M., 1995. Urinary incontinence. Lancet 346, 94–99.

Saito, M., Gotoh, M., Kato, K., Kondo, A., 1991. Influence of age on the rat urinary bladder function. Urol. Int. 47 (Suppl.), 39–42.

Sibley, G.N.A., 1984. A comparison of spontaneous and nerve-mediated activity in bladder muscle from man, pig and rabbit. J. Physiol. 354, 431–443.

Sjogren, C., Andersson, K.E., Husted, S., Mattiasson, A., Moller-Madsen, B., 1982. Atropine resistance of transmurally stimulated isolated human bladder muscle. J. Urol. 128, 1368–1371.

Tong, Y.-C., Hung, Y.-C., Cheng, J.T., 1996. Evidence of nonadrenergic, noncholinergic contraction in rat urinary bladder by 1,1-dimethyl-phenylpiperazinium stimulation in vivo. Eur. Urol. 29, 362–365.

Tong, Y.-C., Hung, Y.-C., Shinozuka, K., Kunitomo, M., Cheng, J.-T., 1997. Evidence of adenosine 5′-triphosphate release from nerve and P_{2x}-purinoceptor mediated contraction during electrical stimulation of rat urinary bladder smooth muscle. J. Urol. 158, 1973–1977.

Zignshin, A.U., Hoyle, C.H.V., Bo, X., Lambrecht, G., Mutschler, E., Bäumert, H.G., Burnstock, G., 1993. PPADS selectively antagonizes P_{2x}-purinoceptor-mediated responses in the rabbit urinary bladder. Br. J. Pharmacol. 110, 1491–1495.