Br. J. Pharmacol. (1993), **109**, 473–479

Functional importance of cholinergic and purinergic neurotransmission for micturition contraction in the normal, unanaesthetized rat

[1]*†Yasuhiko Igawa, *Anders Mattiasson & **Karl-Erik Andersson

Departments of *Urology and **Clinical Pharmacology, Lund University Hospital, Lund, Sweden, and †Department of Urology, Shinshu University School of Medicine, Matsumoto, Japan

1 The cholinergic and purinergic neurotransmission involved in micturition in the normal, unanaesthetized rat was investigated by means of continuous cystometry.

2 ATP (1 and 5 mg kg^{-1}), administered intra-arterially (i.a.) close to the bladder, produced rapid, phasic, dose-dependent increases in bladder pressure with micturition immediately after injection. The micturition pressure of the following spontaneous voidings increased, and bladder capacity, micturition volume, and residual volume decreased. Pretreatment with α,β-methylene ATP (1 mg kg^{-1}, i.a.) blocked the effects of ATP (5 mg kg^{-1}).

3 α,β-Methylene ATP (0.25, 0.5 and 1 mg kg^{-1}, i.a.) produced rapid, phasic, increases in bladder pressure with micturition immediately after injection. The effects of α,β-methylene ATP (0.25 mg kg^{-1}, i.a.) were not affected by pretreatment with indomethacin (0.5–2 mg kg^{-1}, i.a.). The micturition pressure of the subsequent spontaneous voidings decreased, and bladder capacity and residual volume increased.

4 Carbachol (5–50 μg kg^{-1}, i.a.) produced rapid, sustained, dose-dependent increases in bladder pressure with micturition, and then increased basal pressure, threshold pressure, and micturition pressure, and decreased bladder capacity and micturition volume during the following spontaneous voidings.

5 Atropine (1 mg kg^{-1}, i.a.) decreased micturition pressure and micturition volume, but did not induce dribbling incontinence. Micturition contractions still occurred after the injection, but changed in appearance and were of shorter duration than before. In the presence of atropine (1 mg kg^{-1}, i.a.), α,β-methylene ATP (1 mg kg^{-1}, i.a.) produced initially a phasic increase in bladder pressure with micturition and then dribbling incontinence in all animals tested.

6 After blockade of the micturition reflex with morphine (10 μg intrathecally), ATP (5 mg kg^{-1}, i.a.), α,β-methylene ATP (0.25–1 mg kg^{-1}, i.a.), and carbachol (5–500 μg kg^{-1}, i.a.) were unable to empty the bladder.

7 The results suggest that drug-induced bladder emptying in the normal, unanaesthetized rat requires an intact micturition reflex and they support the view that the two physiologically important transmitters involved in micturition are acetylcholine and ATP.

Keywords: Micturition in rat; cholinergic transmission; purinergic transmission; cystometry; α,β-methylene ATP

Introduction

It is generally agreed that reflex activation of the parasympathetic nerve supply to the bladder is responsible for bladder emptying. It is also accepted that acetylcholine is one of the transmitters released during activation, but the well known occurrence of atropine resistance has led to the suggestion that other, non-adrenergic, non-cholinergic (NANC) transmitters may also be involved (Ambache & Zar, 1970). In the rat bladder, for example, the NANC component of contraction seems to be the quantitatively dominating (Carpenter, 1977). Stimulation of NANC-nerves in the guinea-pig bladder evokes contractions that can be mimicked by exogenous adenosine-5′-triphosphate (ATP) and studies on the NANC component of bladder contraction in this and several other species, including rat and man (Burnstock *et al.*, 1972; 1978a,b; Dean & Downie, 1978; Dahlén & Hedqvist, 1980; Kasakov & Burnstock, 1983; Sibley, 1984; Hoyle & Burnstock, 1985; Kasakov & Vlaskova, 1985; Fujii, 1988; Bhat *et al.*, 1989; Brading & Mostwin, 1989; Hoyle *et al.*, 1989; Brading & Williams, 1990; Luheshi & Zar, 1990; Parija *et al.*, 1991) have supported the view that ATP is an excitatory NANC transmitter. The contractile response of guinea-pig, rat, and human detrusor strips to field stimulation was shown to be blocked almost completely by a combination of atropine and desensitization of bladder P_2-purinoceptors with α,β-methylene ATP (Kasakov & Burnstock, 1983; Brading, 1987; Brading & Mostwin, 1989; Hoyle *et al.*, 1989; Brading & Williams, 1990). In the rat bladder, a cholinergic (slow) and a non-cholinergic (fast) contractile component in response to single pulse stimulation could be distinctly separated (Maggi *et al.*, 1985). The slow component was abolished by atropine, and the fast component was reduced, but not abolished, by α,β-methylene ATP (Luheshi & Zar, 1990; Parija *et al.*, 1991; Maggi, 1991). These results suggest that ATP and acetylcholine are the main contractile activators of the rat detrusor.

However, investigations using field stimulation of the nerves of isolated whole bladders or strips of detrusor muscle give no information on the importance of the released transmitters for bladder emptying *in vivo*. In the present investigation, we have studied by continuous cystometry, the cholinergic and the purinergic neurotransmission involved in micturition in normal, unanaesthetized rats.

Methods

Surgical procedures

Female Sprague-Dawley rats weighing 200–250 g were anaesthetized with ketamine (75 mg kg^{-1}, i.m.) and xylazine (15 mg kg^{-1}, i.m.). The abdomen was opened through a mid-

[1] Author for correspondence at: Department of Urology, Lund University Hospital, S-221 85 Lund, Sweden.

line incision, and a polyethylene catheter (Clay-Adams PE-50, NJ, U.S.A.) was implanted into the bladder through the dome as described previously (Malmgren *et al.*, 1987b). Two days after this operation, the animals were again anaesthetized, and a femoral artery was exposed through an inguinal incision. A poleythylene catheter (Clay-Adams PE-10, NJ, U.S.A.) filled with heparinized saline (30 iu ml^{-1}) was inserted into the vessel, and advanced proximally until the tip of the catheter reached the abdominal aortic bifurcation. In order to increase the amount of drug reaching the bladder, both femoral arteries were tied. The catheter was tunnelled subcutaneously and an orifice was made on the back of the animal. In some animals, a polyethylene catheter (Clay-Adams PE-10, NJ, U.S.A.) was implanted into the subarachnoid space at the level of L_6-S_1 spinal cord segments for intrathecal (i.t.) administration of drugs as described in detail previously (Igawa *et al.*, 1992a,b). Neither the placement of the i.t. catheter itself, nor i.t. injection of saline, affects the cystometric pattern (Igawa *et al.*, 1992a,b). After the experiment, the position of the catheter in the abdominal aorta and in the spinal subarachnoid space was confirmed in each animal.

Cystometric investigations

Cystometric investigations were performed without any anaesthesia the day after the intra-arterial (i.a.) catheterization. The bladder catheter was connected via a T-tube to a pressure transducer (P23 DC, Statham Instrument Inc., CA, U.S.A.) and a microinjection pump (CMA 100, Carnegie Medicine AB, Sweden). The conscious rat was placed, without any restraint, in a metabolic cage which also enabled measurements of micturition volumes by means of a fluid collector connected to a Grass force displacement transducer (FT 03C, Grass Instrument Co., Quincy, Mass, U.S.A.). Room-temperature saline was infused into the bladder at a rate of 10 ml h^{-1}. Intravesical pressure and micturition volumes were recorded continuously on a Grass polygraph (Model 7E, Grass Instrument Co., Quincy, Mass, U.S.A.; recording speed: 10 mm min^{-1}). Three reproducible micturition cycles, corresponding to a 20 min period, were recorded before drug administration. After each drug administration, recording was continued for another 60 min. The following cystometric parameters were investigated (Malmgren *et al.*, 1987b): basal pressure (the lowest bladder pressure during filling), threshold pressure (bladder pressure immediately prior to micturition), micturition pressure (the maximum bladder pressure during micturition), bladder capacity (residual volume at the latest previous micturition plus volume of infused saline at the micturition), micturition volume (volume of expelled urine), residual volume (bladder capacity minus micturition volume), and duration of micturition contraction. Analysis was performed for a 20 min period before drug administration. Drug effects on cystometric parameters were assessed for 60 min, and the most effective two micturition cycles were subjected to analysis. When a rapid bladder contraction was observed immediately after drug injection, the amplitude, and duration of the contraction, and percentage volume expelled (i.e. volume of expelled urine/volume of infused saline) were also analysed.

Administration of drugs

The following drugs were used for i.a. or i.t. administration: carbamylcholine chloride (carbachol), adenosine 5'-triphosphate (ATP disodium salt), α,β-methylene ATP (lithium salt), indomethacin (Sigma Chemical Co., St. Louis, MO, U.S.A.), atropine sulphate (Kabi-Pharmacia, Stockholm, Sweden), and morphine sulphate (Gacell Laboratories, Malmö, Sweden). Drugs were dissolved in redistilled water, and then stored at −70°C. Subsequent dilutions of the drugs were made on the day of experiments in saline. Drugs were injected through the i.a. catheter in a volume of 1 ml kg^{-1} (0.20–0.25 ml), and were followed by 0.1 ml of heparinized saline for less than 5 s. To evaluate the micturition induced directly by drugs *per se*, the drugs, except atropine and morphine, were given at the time when the volume of infused saline reached half the bladder capacity during the preceding micturition cycles. Atropine and morphine were injected immediately after micturition. Administration of the vehicles had no effects on the cystometric pattern.

Statistical analysis

The results are given as mean values ± s.e.mean. Student's paired *t* test was used for comparison between before and after treatments. Comparison between the drugs was performed by use of factorial analysis of variance. A probability level <0.05 was accepted as significant.

Results

Repeated cystometries gave reproducible results. The bladder pressure was low and almost devoid of spontaneous fluctuations during filling. The micturition contractions generally had a biphasic appearance (biphasic in 19 out of 23 animals; 83%, monophasic in 4 animals; 17%).

ATP, administered i.a. close to the bladder in a dose of 0.1 mg kg^{-1} ($n = 5$), had no clear-cut effects. In doses of 1 ($n = 9$) and 5 mg kg^{-1} ($n = 10$), ATP produced rapid, phasic, dose-dependent increases in bladder pressure with micturition immediately after the injection. The amplitude and duration of the drug-induced contractions after injection of 5 mg kg^{-1} were 26 ± 6 cmH_2O and 15 ± 2 s, respectively. The percentage volume expelled after 5 mg kg^{-1} was 75 ± 6% (Table 1). The micturition pressure of the following spontaneous voidings increased dose-dependently, and bladder capacity, micturition volume, and residual volume decreased (Figure 1a; Table 2). The duration of the effects was approximately 15 min. Pretreatment with α,β-methylene ATP (1 mg kg^{-1}, i.a., 2–3 min before administration of ATP) blocked the effects of ATP (5 mg kg^{-1}, $n = 6$; Figure 1b).

α,β-Methylene ATP, administered i.a. in doses of 0.25 ($n = 9$), 0.5 ($n = 4$) and 1 mg kg^{-1} ($n = 8$), produced rapid, phasic, dose-dependent increases in bladder pressure with micturition immediately after the injections (Figure 2a). The amplitude and duration of the drug-induced contractions after injection of 0.25 and 1 mg kg^{-1} were 49 ± 8 and 64 ± 3 cmH_2O, and 36 ± 12 and 54 ± 7 s, respectively. The percentage volume expelled after 0.25 and 1 mg kg^{-1} was 76 ± 3 and 77 ± 8%, respectively (Table 1). The effects of α,β-methylene ATP (0.25 mg kg^{-1}, i.a.) were not affected by pretreatment with indomethacin (0.5–2 mg kg^{-1}, i.a.; $n = 5$; data not shown). After injection of 0.25 mg kg^{-1} α,β-methylene ATP, there were no consistent effects on the subsequent spontaneous voidings. After 0.5 and 1 mg kg^{-1} α,β-methylene ATP, on the other hand, the micturition pressure of the subsequent spontaneous voidings decreased, and bladder capacity and residual volume increased (Figure 2a; Table 2). However, dribbling incontinence was not induced. Repeated injections of α,β-methylene ATP (0.25–0.5 mg kg^{-1}, i.a. every 5–7 min × 5–8; $n = 4$) decreased the micturition pressure of the following spontaneous voidings, and changed the micturition contractions in those cases where it was monophasic into a biphasic pattern (Figure 2b). However, the duration of the micturition contractions did not change, and dribbling incontinence was not observed. Five to 10 min after the last of the repeated administrations of α,β-methylene ATP, atropine (1 mg kg^{-1}, i.a.; $n = 4$) was administered. Atropine abolished the second tonic part of the micturition contractions, but did not affect the initial phasic component (Figure 2b). Dribbling incontinence was not produced after the injection of atropine. In 2 animals, α,β-methylene ATP (0.5–1.0 mg kg^{-1}, i.a.) was administered again 10 min after atropine. Small bladder contractions still remained after the injection, but dribbling incontinence was now observed in both animals.

Table 1 The amplitude and duration of the bladder contractions and expelled volume induced by ATP, α,β-methylene ATP, and carbachol given intra-arterially to the rat

	Amplitude (cmH_2O)	*Duration* (s)	*% vol. exp.* (%)
ATP			
5 mg kg^{-1}, $n = 10$	26 ± 6	15 ± 2**	75 ± 6
α,β-Methylene ATP			
0.25 mg kg^{-1}, $n = 9$	49 ± 8	36 ± 12**	76 ± 3
1 mg kg^{-1}, $n = 8$	64 ± 3	54 ± 7**	77 ± 8
1 mg kg^{-1}, $n = 8$ (in the presence of atropine)	22 ± 5†††	24 ± 6††	23 ± 8†††
Carbachol			
5 μg kg^{-1}, $n = 7$	63 ± 16	76 ± 36**	90 ± 4
50 μg kg^{-1}, $n = 8$	65 ± 15	458 ± 108	90 ± 4

Results are expressed as mean ± s.e.mean.
Amplitude; amplitude of the bladder contraction induced by the drugs. Duration; duration of the bladder contraction induced by the drugs. % vol. exp.; percentage volume expelled by the drug-induced bladder contraction compared to carbachol (50 μg kg^{-1}): **$P<0.01$; compared to the corresponding values in the absence of atropine: ††$P<0.01$; †††$P<0.001$.

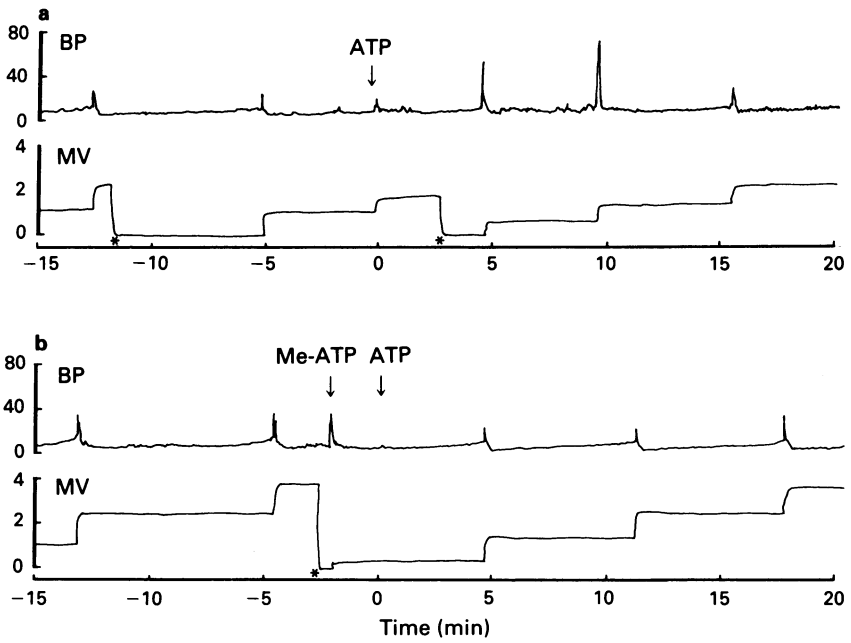

Figure 1 Effects of ATP administered intra-arterially (i.a.) in the absence and presence of α,β-methylene ATP on bladder pressure (BP; cmH_2O) and micturition volume (MV; ml) during cystometry performed in a normal rat. (a) In the absence of α,β-methylene ATP, ATP (5 mg kg^{-1}, i.a.) produced a rapid, phasic rise in bladder pressure with micturition, and then increased micturition pressure during the following spontaneous voidings. (b) α,β-Methylene ATP (Me-ATP; 1 mg kg^{-1}, i.a.), administered 2 min before ATP, by itself produced a rapid, phasic increase in micturition pressure with micturition. In the presence of α,β-methylene ATP, the effects of ATP (5 mg kg^{-1}, i.a.) were blocked. *indicates adjustment to baseline position.

Carbachol, administered i.a. in a dose of 1 μg kg^{-1} ($n = 4$), had no clear-cut effects. Carbachol, in doses of 5 ($n = 7$) and 50 μg kg^{-1} ($n = 8$), produced rapid, sustained, dose-dependent increases in bladder pressure, and micturition (Figure 3). The amplitude and duration of the drug-induced contractions after injection of 5 and 50 μg kg^{-1} were 63 ± 16 and 65 ± 15 cmH_2O, and 76 ± 36 and 458 ± 108 s, respectively (Table 1). The duration of the contraction after carbachol 50 μg kg^{-1} was longer than that after ATP (5 mg kg^{-1}), α,β-methylene ATP (0.25 and 1.0 mg kg^{-1}), and carbachol 5 μg kg^{-1}. The percentage volume expelled after 5 or 50 μg kg^{-1} was 90 ± 4% (Table 1). After 5 and 50 μg kg^{-1}, the basal pressure, threshold pressure, and micturition pressure increased during the following spontaneous voidings. Bladder capacity and micturition volume also decreased (Figure 3; Table 2). The duration of the effects was approximately 15 min.

Atropine (1 mg kg^{-1}, i.a., $n = 9$) decreased micturition pressure (by 54%) and micturition volume, and increased residual volume for about 30 min, but did not induce dribbling incontinence (Figure 4; Table 2). Micturition contractions still occurred after the injection, but changed appearance (biphasic in 7, monophasic in 2 out of 9 animals before administration, monophasic in 9 out of 9 animals after administration) and were of shorter duration than before (Figure 4). The mean duration of the micturition contraction after administration was 6.0 ± 0.6 s, which was significantly shorter ($P<0.001$) than the corresponding value (12.0 ± 0.6 s) before administration. In the presence of atropine (1 mg kg^{-1}, i.a., 8–15 min before administration of α,β-methylene ATP), α,β-methylene ATP (1 mg kg^{-1}, i.a.; $n = 8$) produced initially a phasic increase in bladder pressure with micturition. The amplitude and duration of the drug-induced contraction after injection of α,β-methylene were

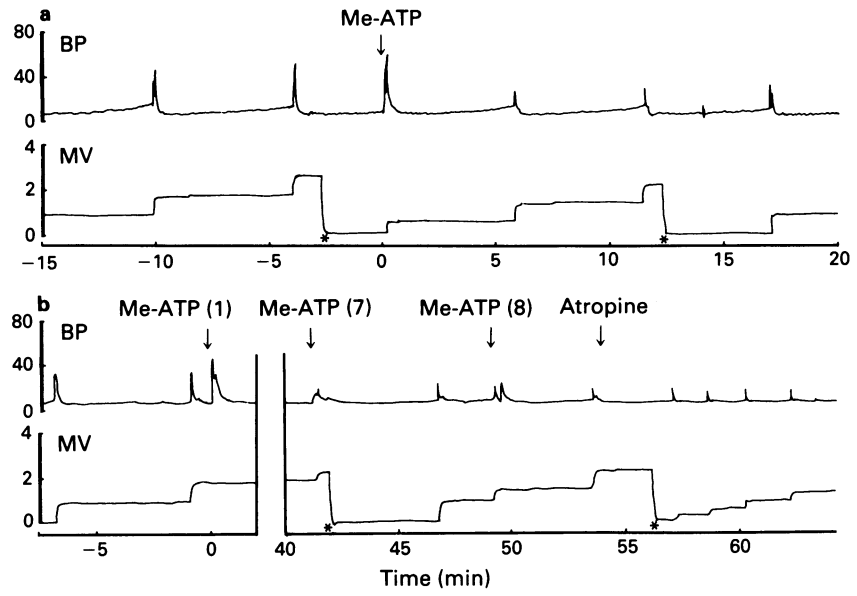

Figure 2 Original recordings of bladder pressure (BP; cmH_2O) and micturition volume (MV; ml) during cystometry performed in a normal rat before and after intra-arterial (i.a.) single injection (a), and repeated injections (b) of α,β-methylene ATP. (a) α,β-Methylene ATP (Me-ATP; 1 mg kg^{-1}, i.a.) induced a phasic pressure rise with micturition, and suppressed the following micturition contractions. (b) Repeated administration of α,β-methylene ATP (Me-ATP; 0.5 mg kg^{-1}, i.a., 8 times, 7 min intervals) produced a decrease in micturition pressure, and changed the micturition contractions from a mono- to a biphasic pattern. Atropine (1 mg kg^{-1}, i.a.) abolished the second tonic component of the biphasic contraction. *indicates adjustment to baseline position. Number of α,β-methylene ATP administrations given in parentheses.

Table 2 Effects of intra-arterial administration of ATP (5 mg kg^{-1}, $n = 10$), α,β-methylene ATP (1 mg kg^{-1}, $n = 8$), carbachol (50 μg kg^{-1}, $n = 8$), and atropine (1 mg kg^{-1}, $n = 9$) on cystometric parameters during the following spontaneous voidings

	MP	*BC*	*MV*	*RV*
ATP				
before	47.8 ± 5.7	1.15 ± 0.08	1.03 ± 0.08	0.11 ± 0.02
after	73.9 ± 9.6***	0.83 ± 0.09***	0.78 ± 0.08***	0.05 ± 0.02*
α,β-Methylene ATP				
before	54.2 ± 5.8	0.82 ± 0.07	0.76 ± 0.06	0.06 ± 0.02
after	30.8 ± 5.8**	1.04 ± 0.09*	0.66 ± 0.07	0.37 ± 0.04***
Carbachol				
before	44.0 ± 9.6	1.00 ± 0.11	0.94 ± 0.09	0.06 ± 0.04
after	62.0 ± 12.9*	0.41 ± 0.06***	0.39 ± 0.05***	0.02 ± 0.01
Atropine				
before	46.0 ± 4.0	0.80 ± 0.08	0.75 ± 0.08	0.05 ± 0.02
after	21.1 ± 3.3***	0.95 ± 0.11	0.48 ± 0.02***	0.47 ± 0.09***

MP: micturition pressure (cmH_2O); BC: bladder capacity (ml); MV: micturition volume (ml); RV residual volume (ml).
Results are expressed as mean ± s.e.mean.
Values before vs after administration: *$P<0.05$; **$P<0.01$; ***$P<0.001$.

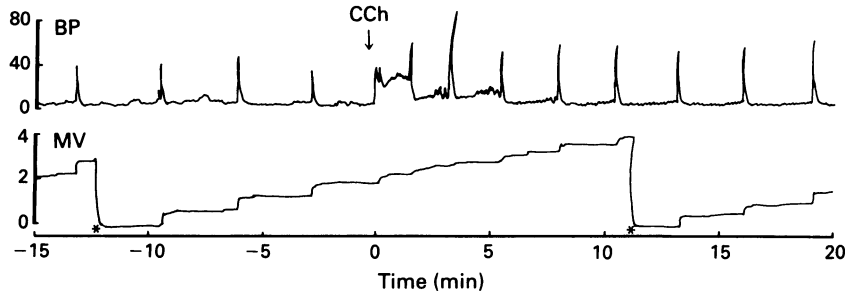

Figure 3 Effects of carbachol administered intra-arterially on bladder pressure (BP; cmH_2O) and micturition volume (MV; ml) during cystometry performed in a normal rat. Immediately after injection of carbachol (CCh; 50 μg kg^{-1}), a rapid, sustained increase in bladder pressure with micturition was observed. Then, the basal, threshold, and micturition pressures increased, and micturition volume decreased during the following spontaneous voidings. *indicates adjustment to baseline position.

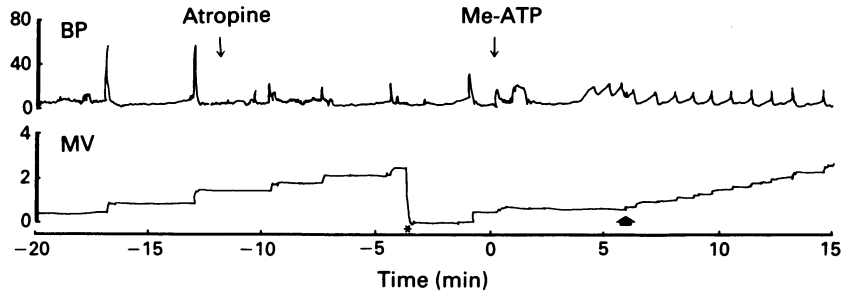

Figure 4 Effects of atropine, and α,β-methylene ATP in the presence of atropine on bladder pressure (BP; cmH_2O) and micturition volume (MV; ml) during cystometry performed in a normal rat. Atropine (1 mg kg^{-1}), administered intra-arterially (i.a.), by itself decreased micturition pressure and micturition volume, and shortened the micturition contraction. In the presence of atropine, α,β-methylene ATP (Me-ATP; 1 mg kg^{-1}, i.a.) produced a rapid, transient increase in bladder pressure, and then dribbling incontinence. * and ↑ indicate adjustment to baseline position and initiation of dribbling incontinence, respectively.

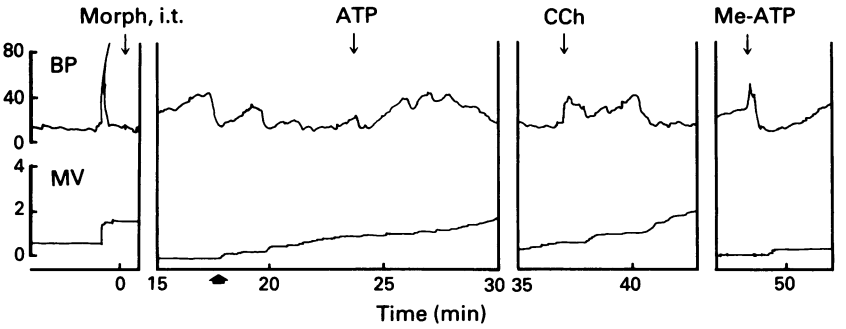

Figure 5 Effects of carbachol, ATP, and α,β-methylene ATP, administered intra-arterially (i.a.), in the presence of morphine on bladder pressure (BP; cmH_2O) and micturition volume (MV; ml) during cystometry performed in a normal rat. Morphine, administered intrathecally (Morph. i.t.; 10 μg), blocked micturition contractions and produced dribbling incontinence. Carbachol (CCh; 50 μg kg^{-1}, i.a.) ATP (5 mg kg^{-1}, i.a.), and α,β-methylene ATP (Me-ATP; 1 mg kg^{-1}, i.a.) produced small increases in bladder pressure, but did not induce micturition. ↑ indicates initiation of dribbling incontinence.

22 ± 5 cmH_2O and 24 ± 6 s, respectively. The percentage volume expelled was $23 \pm 8\%$ (Table 1). The micturition contractions were abolished in 3 animals out of 8, and the amplitude of the bladder pressure increase was reduced from 18.6 ± 2.4 to 7.6 ± 2.6 cmH_2O ($P < 0.05$) in the remaining 5 animals. Dribbling incontinence was observed in all animals (Figure 4).

Intrathecal administration of morphine (10 μg) blocked micturition contractions and produced dribbling incontinence, which persisted for more than 2 h. In the presence of morphine (10 μg, i.t.), carbachol (5–500 μg kg^{-1}, i.a.; $n = 6$), ATP (5 mg kg^{-1}, i.a.; $n = 6$), and α,β-methylene ATP (0.25–1 mg kg^{-1}, i.a.; $n = 6$) were all unable to produce bladder emptying, i.e. the percentage volume expelled was less than 10%, even if bladder contractions were noted after the injections (Figure 5).

Discussion

The present study showed that ATP and α,β-methylene ATP were able to induce bladder contraction and micturition in the normal, unanaesthetized rat. Even when the purinoceptors were desensitized by repeated exposure to α,β-methylene ATP, the micturition contraction, although decreased, was not abolished. This is in line with the results of several other investigations supporting the view that ATP is a NANC transmitter in the rat (Dahlén & Hedqvist, 1980; Brading & Williams, 1990; Luheshi & Zar, 1990; Parija *et al.*, 1991), but casts some doubt about its functional predominance under *in vivo* conditions (cf. *in vitro*; Carpenter, 1977). Also carbachol induced bladder contractions leading to bladder emptying. The volumes expelled by these contractions were about 75% of the bladder content after ATP or α,β-methylene ATP, and 90% after carbachol. These results suggest that not only cholinergic but also purinergic transmission is of importance for both pressure generation and emptying of the bladder *in vivo* in rats.

Based on their studies on field-stimulated rabbit and cat whole bladder preparations *in vitro*, Levin *et al.* (1986; 1990) and Chancellor *et al.* (1992) suggested that non-cholinergic transmission may initiate bladder contraction, but that the ability to empty the bladder in these two species is primarily a function of cholinergic transmission. This conclusion may be valid also for the rat, but factors not possible to study in the *in vitro* preparation come into play *in vivo*. Thus, Chancellor *et al.* (1992) found ATP unable to empty the rabbit isolated bladder with moderate outlet resistance, whereas bethanechol and electrical field stimulation could. *In vivo*, we found that exogenous ATP could empty the bladder. Downie *et al.* (1983) found that the ability of bethanechol to empty the bladder in cats was dependent on an intact micturition reflex. This may also be the case for the contractions induced by ATP, α,β-methylene ATP, and carbachol in the rat. When the micturition reflex was blocked by morphine administered i.t., none of these agents produced bladder emptying. Durant & Yaksh (1988) also found that neither bethanechol, nor α-adrenoceptor agonists, were able to empty the bladder when micturition was blocked by i.t. morphine. These findings suggest that an intact micturition reflex is necessary for efficient bladder emptying *in vivo*. It may be that both ATP, α,β-methylene ATP, and carbachol, when administered *in vivo*, by increasing tone of the detrusor muscle cells, elicit a micturition reflex leading to synchronized detrusor contraction and outlet relaxation with consequent bladder emptying. An increase in bladder pressure alone does not seem to be sufficient for bladder emptying *in vivo*. A direct stimulating effect of bethanechol on the central nervous system (locus caeruleus) has been suggested also to contribute to initiation of micturition (O'Donnell, 1990; Stephenson, 1991). This may also be the case for carbachol.

As shown previously in the rat (Malmgren *et al.*, 1987a), atropine did not abolish the micturition contractions. However, atropine changed their characteristics. They became more rapid and shortlasting than previously, and similar to the contractions induced by ATP. The bladder contraction induced by carbachol lasted longer than that induced by ATP or α,β-methylene ATP. In addition, repeated injections of α,β-methylene ATP, which antagonized the effects of ATP, reduced the amplitude of the micturition contractions, and disclosed a second, tonic component of the contraction. The second component, but not the first, was abolished by atropine. This is in agreement with findings in rat isolated detrusor strips (Maggi *et al.*, 1985; Parija *et al.*, 1991), which responded to single pulse stimulation with a biphasic contractile response consisting of an initial, fast, and an ensuing slow component. α,β-Methylene ATP abolished ATP-induced contractions, and inhibited the fast component by 38%, but did not alter the slow component, whereas atropine selectively abolished the slow component. Also in the cat, Theobald (1983) reported two distinct bladder responses to parasympathetic nerve stimulation. The initial response was a sharp, transient rise in intravesical pressure, and the second consisted of a tonic maintenance during stimulation. The photoaffinity analogue of ATP, $ANAPP_3$, which blocked ATP-induced contractions, partially blocked the first phase, and atropine, which had no effect on the first response, blocked the second phase of the contraction.

The observation that the contractile responses of guinea-pig and rat detrusor strips to field stimulation was blocked to a minor extent when atropine and α,β-methylene ATP were given separately, but was almost abolished by a combination of the two drugs (Brading, 1987; Brading & Mostwin, 1989; Brading & Williams, 1990), suggests that interaction between the two transmitters may be important for normal micturition. This view was supported by the present study, since in the normal, unanaesthetized rat, the combination of atropine and desensitization of bladder P_2-purinoceptors with α,β-methylene ATP induced retention and dribbling incontinence. However, neither atropine, nor α,β-methylene ATP, when given alone, produced dribbling incontinence in any animal tested.

Even if this and previous studies support a role for ATP in NANC neurotransmission, ATP may not be the sole NANC transmitter. Based on their findings in rabbit and guinea-pig bladders, Creed *et al.* (1991) suggested that either the desensitization with α,β-methylene ATP is not complete, or other NANC nerves contribute to the innervation. Luheshi & Zar (1990) found that the responses to electrical stimulation in the rat bladder were reduced following α,β-methylene ATP desensitization, but that a sizable proportion of the response persisted in the presence of atropine and indomethacin. They concluded therefore that ATP is unlikely to be the sole non-cholinergic motor transmitter in the rat detrusor. Choo & Mitchelson (1980), using ATP as the desensitizing nucleotide, had previously arrived at a similar conclusion.

The present study demonstrated that in the presence of atropine, α,β-methylene ATP produced dribbling incontinence, even if some animals still showed small bladder contractions, which did not lead to bladder emptying. On the other hand, atropine added 5–10 min after repeated injections of α,β-methylene ATP did not produce dribbling incontinence. When α,β-methylene ATP was given again after atropine to these animals, dribbling incontinence was induced. Considering the short duration of action of α,β-methylene ATP, and the fact that the maximum action of atropine was seen after 5–10 min, the possibility cannot be excluded that the contractions remaining after desensitization with α,β-methylene ATP and addition of atropine were mediated via purinoceptors. However, even if transmitters other than acetylcholine and ATP may be released from bladder nerves, their functional relevance for bladder emptying may be questioned, at least in the rat. Obviously, when these two main transmitters were blocked, no effective emptying contraction could be elicited, resulting in dribbling incontinence in all animals tested.

In conclusion, the results of the present study suggest that drug-induced bladder emptying in the normal, unanaesthetized rat requires an intact micturition reflex, and they support the view that the two physiologically important transmitters involved in micturition are acetylcholine and ATP.

This study was supported by the Swedish Medical Research Council (grants no. 6837 & 10399), and the Medical Faculty, University of Lund.

References

AMBACHE, N. & ZAR, M.A. (1970). Non-cholinergic transmission by post-ganglionic motor neurones in the mammalian bladder. *J. Physiol.*, **210**, 761–783.

BHAT, M.B., MISHRA, S.K. & RAVIPRAKASH, V. (1989). Differential susceptibility of cholinergic and noncholinergic neurogenic responses to calcium channel blockers and low Ca^{2+} medium in rat urinary bladder. *Br. J. Pharmacol.*, **96**, 837–842.

BRADING, A.F. (1987). The physiology of bladder smooth muscle. In *The Physiology of the Lower Urinary Tract*. ed. Torrens, M. & Morrison, J. pp. 161–191. Berlin: Springer-Verlag.

BRADING, A.F. & MOSTWIN, J.L. (1989). Electrical and mechanical responses of guinea-pig bladder muscle to nerve stimulation. *Br. J. Pharmacol.*, **98**, 1083–1090.

BRADING, A.F. & WILLIAMS, J.H. (1990). Contractile responses of smooth muscle strips from rat and guinea-pig urinary bladder to transmural stimulation: effects of atropine and α,β-methylene ATP. *Br. J. Pharmacol.*, **99**, 493–498.

BURNSTOCK, G., COCKS, T., CROWE, R. & KASAKOV, L. (1978a). Purinergic innervation of the guinea-pig urinary bladder. *Br. J. Pharmacol.*, **63**, 125–138.

BURNSTOCK, G., COCKS, T., KASAKOV, L. & WONG, A. (1978b). Direct evidence for ATP release from non-adrenergic, non-cholinergic ('purinergic') nerves in the guinea-pig taenia coli and bladder. *Eur. J. Pharmacol.*, **49**, 145–149.

BURNSTOCK, G., DUMSDAY, B. & SMYTHE, A. (1972). Atropine-resistant excitation of the urinary bladder: the possibility of transmission via nerves releasing a purine nucleotide. *Br. J. Pharmacol.*, **44**, 451–461.

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

CHANCELLOR, M.B., KAPLAN, S.A. & BLAIVAS, J.G. (1992). The cholinergic and purinergic components of detrusor contractility in a whole rabbit bladder model. *J. Urol.*, **148**, 906–909.

CHOO, L.K. & MITCHELSON, F. (1980). The effect of indomethacin and adenosine 5'-triphosphate on the excitatory innervation of the rat urinary bladder. *Can. J. Physiol. Pharmacol.*, **58**, 1042–1048.

CREED, K.E., ITO, Y. & KATSUYAMA, H. (1991). Neurotransmission in the urinary bladder of rabbits and guinea pigs. *Am. J. Physiol.*, **261**, C271–C277.

DAHLÉN, S.E. & HEDQVIST, P. (1980). ATP, β,γ-methylene ATP and adenosine inhibit non-cholinergic, non-adrenergic transmission in the rat urinary bladder. *Acta Physiol. Scand.*, **109**, 137–142.

DEAN, D.M. & DOWNIE, J.W. (1978). Contribution of adrenergic and 'purinergic' neurotransmission to contraction in the rabbit detrusor. *J. Pharmacol. Exp. Ther.*, **207**, 431–445.

DOWNIE, J.W., MOOCHALA, S.M. & BIALIK, G.J. (1983). The role of reflexes in modifying the response to bethanechol chloride: a basis for selectivity of action on the bladder. *Neurourol. Urodyn.*, **2**, 301–309.

DURANT, P.A.C. & YAKSH, T. (1988). Drug effects on urinary bladder tone during spinal morphine-induced inhibition of the micturition reflex in unanaesthetized rats. *Anesthesiol.*, **68**, 325–334.

FUJII, K. (1988). Evidence for adenosine triphosphate as an excitatory transmitter in guinea-pig, rabbit and pig urinary bladder. *J. Physiol.*, **404**, 39–52.

HOYLE, C.H.V. & BURNSTOCK, G. (1985). Atropine-resistant excitatory junction potential in rabbit bladder are blocked by α,β-methylene ATP. *Eur. J. Pharmacol.*, **114**, 239–240.

HOYLE, C.H.V., CHAPPLE, C. & BURNSTOCK, G. (1989). Isolated human bladder: evidence for an adenosine dinucleotide acting on P_{2x}-purinoceptors and for purinergic transmission. *Eur. J. Pharmacol.*, **174**, 115–118.

IGAWA, Y., ANDERSSON, K.-E., POST, C., UVELIUS, B. & MATTIASSON, A. (1992a). A rat model for investigation of spinal mechanisms in detrusor instability associated with infravesical outflow obstruction. *J. Urol.*, **147**, 349A (abstract no. 546).

IGAWA, Y., PERSSON, K., ANDERSSON, K.-E., UVELIUS, B. & MATTIASSON, A. (1992b). Facilitatory effect of vasoactive intestinal polypeptide on spinal and peripheral micturition reflex pathways in conscious rats with and without detrusor instability. *J. Urol.*, (in press).

KASAKOV, L. & BURNSTOCK, G. (1983). The use of the slowly degradable analogue, α,β-methylene ATP to produce desensitisation of the P_2-purinoceptor: effect on non-adrenergic, non-cholinergic responses of the guinea-pig urinary bladder. *Eur. J. Pharmacol.*, **86**, 291–294.

KASAKOV, L. & VLASKOVA, M.V. (1985). Profile of prostaglandins generated in the detrusor muscle of rat urinary bladder: effects of adenosine triphosphate and adenosine. *Eur. J. Pharmacol.*, **113**, 431–436.

LEVIN, R.M., LONGHURST, P.A., KATO, K., MCGUIRE, E.J., EL-BADAWI, A. & WEIN, A.J. (1990). Comparative physiology and pharmacology of the cat and rabbit urinary bladder. *J. Urol.*, **143**, 848–852.

LEVIN, R.M., RUGGIERI, M.R. & WEIN, A.J. (1986) Functional effects of purinergic innervation of the rabbit urinary bladder. *J. Pharmacol. Exp. Ther.*, **236**, 452–460.

LUHESHI, G. & ZAR, A. (1990). Purinoceptor desensitization impairs but does not abolish the non-cholinergic motor transmission in rat isolated urinary bladder. *Eur. J. Pharmacol.*, **185**, 203–208.

MAGGI, C.A. (1991). Omega conotoxin and prejunctional modulation of the biphasic response of the rat isolated urinary bladder to single pulse electrical field stimulation. *J. Autonom. Pharmacol.*, **11**, 295–304.

MAGGI, C.A., SANTICIOLI, P. & MELI, A. (1985). Pharmacological evidence for the existence of two components in the twitch response to field stimulation of detrusor strips from the rat urinary bladder. *J. Autonom. Pharmacol.*, **5**, 221–230.

MALMGREN, A., SJÖGREN, C., ANDERSSON, K.-E. & ANDERSSON, P.O. (1987a). Effects of atropine on bladder capacity and instability in rats with bladder hypertrophy. *Neurourol. Urodyn.*, **6**, 331–338.

MALMGREN, A., SJÖGREN, C., UVELIUS, B., MATTIASSON, A., ANDERSSON, K.-E. & ANDERSSON, P.O. (1987b). Cystometrical evaluation of bladder instability in rats with infravesical outflow obstruction. *J. Urol.*, **137**, 1291–1294.

O'DONNELL, P.D. (1990). Central actions of bethanechol on the urinary bladder in dogs. *J. Urol.*, **143**, 634–637.

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.

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.

STEPHENSON, J.D. (1991). Pharmacology of the central control of micturition. *Funct. Neurol.*, **6**, 211–217.

THEOBALD JR., R.T. (1983). The effect of arylazido aminopropionyl ATP on atropine resistant contractions of the cat urinary bladder. *Life Sci.*, **32**, 2479–2484.

(Received November 20, 1992
Revised January 4, 1993
Accepted February 8, 1993)