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Effect on urinary bladder function and arterial blood pressure of the activation of putative purine receptors in brainstem areas.

Isabel Rocha [1], Geoffrey Burnstock, K. Michael Spyer *

Autonomic Neuroscience Institute, Department of Physiology, Royal Free and University College Medical School, Rowland Hill Street, London NW3 2PF, UK

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Abstract

The effect on bladder function and arterial blood pressure of adenosine-5′-triphosphate (ATP) and its synthetic analogue, α,β-methylene ATP (α,β-meATP) applied by microinjection to brainstem areas was assessed in the anaesthetised, paralysed and artificially ventilated female rat. Recordings of bladder pressure, changes in the pelvic nerve activity, arterial blood pressure and heart rate were evaluated. The purinergic drugs were microinjected into two brainstem areas – the periaqueductal grey matter (PAG) and the area of the Barrington nucleus/locus coeruleus (LC) – only after electrical stimulation (50 Hz, 1 ms, 30–50 μA; $n_{(PAG)} = 17$; $n_{(LC)} = 18$) and the microinjection of glutamate (2 mM, pH 7.4 ± 0.1; $n_{(PAG)} = 16$; $n_{(LC)} = 16$) had shown increases of bladder pressure and/or rate of bladder contractions and/or pelvic nerve activity at specific sites. Electrical and glutamate activation of PAG evoked an increase of arterial blood pressure. Microinjections of ATP (20 mM, pH 7.4 ± 0.1; $n_{(PAG)} = 11$; $n_{(LC)} = 11$) and α,β-meATP (2 mM, pH 7.4 ± 0.1; $n_{(PAG)} = 10$; $n_{(LC)} = 9$) both evoked consistent increases of bladder pressure and/or pelvic nerve activity. Stimulation with ATP elicited a biphasic change of arterial blood pressure characterised by an increase followed by a decrease which was accompanied by a rise of heart rate. Microinjection of α,β-meATP into PAG did not elicit a consistent response: a decrease of arterial blood pressure was evoked in five rats, while in two other rats an increase occurred. Electrical stimulation and glutamate activation of Barrington's nucleus/LC evoked an increase of arterial blood pressure, but a decrease was observed after microinjection of both ATP and α,β-meATP. At some sites ($n = 8$) the effect of α,β-meATP after a pre-injection at the same site of the P2 purino receptor antagonist, suramin (20 mM, pH 7.4 ± 0.1) was smaller than the control. At three sites within PAG and two within LC located more medially to sites where an excitatory response had been observed, electrical stimulation evoked a small decrease or no change in bladder pressure. Following the stimulus, a rise in bladder pressure was preceded by an increase of pelvic nerve activity. A similar effect of glutamate was observed in one case. These data suggest that activation of P2 purine receptors in both PAG and Barrington's nucleus/LC is implicated in the neuronal mechanisms that generate patterns of activity in the parasympathetic innervation of the bladder and that purines also act at this level to modify sympathetic outflow to the cardiovascular system.

Keywords: Urinary bladder; Blood pressure; Glutamate; ATP; α,β-meATP; Electrical stimulation; PAG; Locus coeruleus; Barrington's nucleus; Midbrain

1. Introduction

The nervous control of the urinary bladder and both the internal and external urethral sphincters, appears to be organised at several levels of the central nervous system (CNS) but the extent, organisation and interactions of these supraspinal centres are not as yet well defined. Several studies both anatomical and electrophysiological have stressed the importance of a rostral brainstem area known as pontine micturition centre (PMC) in the co-ordination of urinary bladder and urethral sphincter activity. This area appears to participate in a reflex pathway, which is triggered by mechanoreceptor afferents, activated by bladder distension. (Torrens and Morrison, 1987; Burnstock, 1990; Kruse et al., 1990). Several attempts have been made to identify the PMC using techniques such as lesioning, axonal tracing or electrophysiology. In the rat, the PMC has been localised in a region just medial to the locus coeruleus (LC) often termed Barrington's nucleus (Hols-

* Corresponding author. Tel.: +44-20-7830-2764; fax: +44-20-7794-3505.

E-mail address: k.spyer@ucl.ac.uk (K.M. Spyer).

[1] Present address: Instituto de Fisiologia, Faculdade de Medicina de Lisboa, Av. Prof. Egas Moniz, 1649-028 Lisbon, Portugal.

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tege et al., 1979, 1986; Paxinos and Watson, 1986; Holstege and Tan, 1987; Sugaya and Matsuyama, 1987; Noto et al., 1991; de Groat et al., 1993) whilst in the cat, the PMC has been localised within the LC, extending from the LCα (Holstege and Tan, 1987) to include an additional population of neurones located ventromedial to the LC adjacent to the mesencephalic tract of the trigeminal nerve (de Groat et al., 1993).

However, other regions of the pons and mesencephalon appear to have a role in the regulation of bladder activity. Anterograde tracing studies involving injection of tracers into the sacral spinal cord, despite showing projections into the dorsal pontine tegmentum, also demonstrated projections into the periaqueductal grey matter (PAG; de Groat et al., 1993). More recently, Ding et al. (1997) identified the ventrolateral division of PAG as receiving projection fibres from the lumbosacral cord and sending axons to Barrington's nucleus, suggesting that PAG may play an important role in controlling bladder activity in the rat. In relation to these anatomical data, an electrophysiological study in the rat by Noto et al. (1991) showed that electrical stimulation of bladder afferents evoked negative field potentials in the dorsal part of PAG at latencies of 13–15 ms but evoked negative field potential with a much longer latency (35–40 ms) in the region of the lateral dorsal tegmental nucleus (LTD) (Barrington's pontine micturition centre). Furthermore, electrical stimulation at dorsal sites in the lateral parabrachial nucleus and the PAG facilitates bladder activity (Torrens and Morrison, 1987; Burnstock, 1990). These data support speculation that PAG may receive spinomesencephalic inputs carrying afferent information from the urinary bladder and that neurons in PAG may, in turn, relay this information to the LTD.

Experiments by Watkins (1938) showed that bladder distension evoked increases in arterial blood pressure (BP) in animals with intact spinal cord. In man, some authors (Robertson and Wolff, 1950; Lapides and Lovegrove, 1965; Szasz and White, 1967) have concluded that bladder distension does not evoke changes in BP or in heart rate (HR) at normal fullness levels and Lapides and Lovegrove (1965) found that pressure responses to bladder distension were obtained only when accompanied by pain. On the other hand, Garnier and Gloor (1967) also showed increases in HR and BP in humans during bladder distension but observed that the rise in arterial pressure started early during bladder filling, refuting the idea that the elevation of pressure was mainly related to pain. In cat, Daly and co-workers (Daly et al., 1982, 1986) showed a consistent tachycardia, an increase in BP and hind-limb vasoconstriction on bladder distension. Recordings of postganglionic sympathetic nerve activity taken from renal, splenic and external carotid nerves showed that large degrees of bladder distension evoked increases in activity in all three nerves (Weaver, 1985). However, small distension only evoked increases in the activity of the external carotid nerve.

Aside from the anatomical localisation of the micturition reflex pathway, there has been considerable interest in defining the neurotransmitters involved in the reflex pathways. γ-Aminobutyric acid (GABA), glycine and enkephalins appear to have inhibitory functions in the micturition reflex whereas glutamic acid or glutamate analogues enhance urinary bladder activity in the cat, and in the rat when injected in the LC and in the parabrachial nucleus (de Groat et al., 1993). Additional data has accumulated suggesting a role of adenosine 5′-triphosphate (ATP) as a neurotransmitter, or modulator, in the CNS (see Abbrachio, 1997). Its effects in the CNS are largely mediated via P2 purine receptors and we have became interested in their potential role in the micturition reflex pathway and in the modulation of arterial blood pressure given their P2 receptor distribution in regions of the brainstem connected with the autonomic function (Jahr and Jessel, 1983; Sun et al., 1992; Tschöpl et al., 1992; Rocha et al., 1997).

In this study, we have assessed the effects of applying ATP and its synthetic analogue α,β-methylene ATP (α,β-meATP) in LC region and PAG by microinjection at sites where electrical stimulation and microinjections of glutamic acid evoked an excitatory effect on bladder function and an increase on arterial blood pressure. A preliminary report of part of this study was communicated to the British Physiological Society (Rocha et al., 1997).

2. Methods

2.1. Surgical procedures

Experiments were performed in 25 female Sprague–Dawley rats (aged more than 10 weeks) anaesthetised with sodium pentobarbitone (Sagatal, 40 mg/kg, i.p.) supplemented as necessary. The femoral artery and vein were cannulated for monitoring arterial blood pressure and the administration of drugs, respectively. The trachea was cannulated below the larynx and the animal was ventilated with O_2-enriched air applied after paralysis with gallamine triethiodide (Flaxedil, 4 mg/kg/h) using a positive pressure ventilator (Harvard Apparatus Ltd). An adequate level of anaesthesia was maintained by ensuring the absence of a withdrawal reflex before paralysing the animal and alterations of blood pressure and heart rate to pinching a paw after the paralysis. Rectal temperature was kept at 36.5–38°C by a servo controlled heating blanket (Harvard Apparatus Ltd). The electrocardiogram (ECG) was recorded (Neurolog, Digitimer Ltd) from needle-electrodes inserted into the limbs.

A mid-line abdominal incision was made to expose the pelvic viscera. Both ureters were ligated distally. The urinary bladder was cannulated through the external urethra for measuring urinary bladder pressure and the injection of warm saline. The right pelvic ganglion was identi-

fied and the right pelvic nerve was dissected and placed on a bipolar silver recording electrode. The signal of nerve activity was amplified and filtered (Neurolog, Digitimer Ltd.) and displayed on an oscilloscope. In three animals pelvic nerve fibres were cut distally and all the experimental procedures were repeated.

The animal's head was placed in a stereotaxic frame (Kopf Instruments) such that the difference in height between lambda and bregma was zero. A craniotomy was carried out to allow the insertion of stimulation electrodes.

2.2. Experimental protocol

At the beginning of each experiment the urinary bladder was filled with warm saline until bladder pressure was raised sufficiently to evoke one to two bladder contractions of 30 cmH_2O per min. The bladder was then partially emptied to restore a low level of bladder pressure (8–16 cmH_2O). In this situation, contractions of small amplitude were observed (2–4 cmH_2O; Fig. 1A) often preceded by increases in pelvic nerve activity. Occasionally, two closely consecutive spontaneous bursts of nerve activity evoked a higher amplitude contraction (25–27 cmH_2O; Fig. 1B).

A multi-barrelled glass microelectrode (tip diameter 40–60 μm) was inserted into the right or left LC region and into the right or left PAG using stereotaxic co-ordinates (Paxinos and Watson, 1986). The barrels of the microelectrode were filled with Wood's metal for electrical stimulation (50 Hz, 1 ms, 30–50 μA), glutamic acid (2 mM, pH 7.4 ± 0.1), ATP (20 mM, pH 7.4 ± 0.1), α,β-meATP (2 mM; pH 7.4 ± 0.1) and suramin, a P2 antagonist (20 mM; pH 7.4 ± 0.1). The last barrel was filled with pontamine sky blue dye (2%) in sodium acetate 1 M in order to mark the sites of stimulation. The microinjection of ATP (50 nl) and α,β-meATP (50 nl) was performed at the sites where a previous electrical stimulus (50 Hz, 1 ms, 30–50 μA, trains of 5s) and microinjection of glutamate (50 nl) had evoked increases in bladder pressure, frequency of bladder contractions and/or pelvic nerve activity.

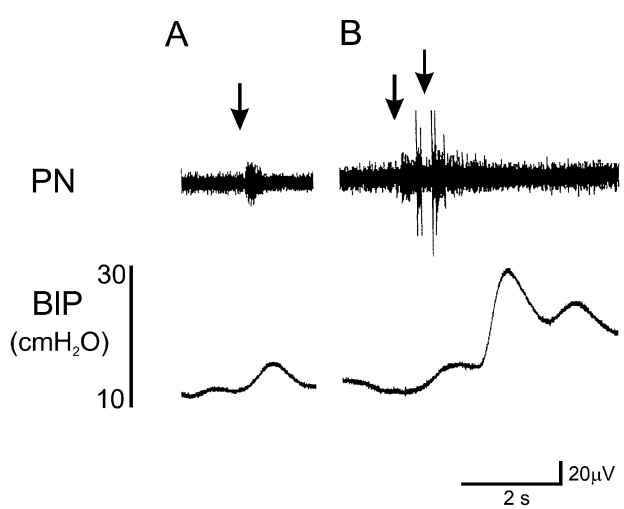

Fig. 1. (A) Contractions of small amplitude were observed after emptying the urinary bladder. These contractions were preceded by an increase of pelvic nerve activity (PN). (B) Occasionally, bursts of nerve activity evoke a higher amplitude contraction. This is an example of the effect of a sumation of two bursts of nerve activity on bladder contractions; BlP, urinary bladder pressure.

In order to investigate the specificity of the response on bladder pressure and pelvic nerve activity, suramin, a P2 antagonist, was also microinjected at the sites where an injection of ATP or α,β-meATP had been effective. The microinjection of the agonist was then repeated after 5 min. Also, glutamate microinjections were performed after the injection of suramin as a control for the non-selectivity of suramin action. The volume of each injection (50 nl) was controlled using a microscope with a calibrated graticule. Saline (0.9%) was used for vehicle control injections.

2.3. Drugs

All drugs were dissolved in 0.9% saline and freshly prepared. The drugs and their sources were ATP (adenosine 5′-triphosphate, disodium salt, Sigma Chemical Co.), α,β-meATP (α,β-methylene adenosine 5′-triphosphate, lithium salt, Sigma Chemical Co.), glutamic acid (monosodium salt, Sigma Chemical Co.) and suramin (suramin hexasodium, Research Biochemical Inc.).

2.4. Histological procedures

Stimulation sites were marked with a microinjection of pontamine blue dye and subsequently identified. The brain was removed, fixed in 4% para-formaldehyde saline with 30% sucrose for 48 h. The tissue was then sectioned serially (80 μm) and the slices stained with neutral red. The location of the stimulation sites was confirmed under a light microscope according to stereotaxic co-ordinates (Paxinos and Watson, 1986).

2.5. Data analysis

All recorded variables were digitised (Instrutech VR100B, Digitimer Ltd.) and recorded on videotape. Off-line analysis was done using a computer A/D system with data capture and analysis software (CED1401, Spike2). For statistical analysis the paired *t*-test was used and differences were considered significant where $P < 0.05$.

All data are expressed as mean ± S.E.M. For the recorded arterial blood pressure and heart rate, the baseline values were taken immediately before the beginning of either an electrical or a chemical stimulation. The baseline values for bladder pressure were taken as a mean from the 10 s before the stimulus in the case of stable recordings and 50 s in the case of unstable recordings. These values were compared with the peak of the response evoked by each type of stimulation.

3. Results

3.1. Locus coeruleus region

3.1.1. Effect of chemical stimulation with glutamate

The microinjection of glutamate produced an increase of bladder pressure from 11.8 ± 0.52 to 38.6 ± 1.52 cmH_2O ($n = 16$; $t = 15.52$; $P < 0.0001$; Fig. 2A, C) and an increase of BP from 99.5 ± 1.32 to 113.6 ± 1.33 mmHg ($n = 11$; $t = 11.29$; $P < 0.0001$; Fig. 2B, C) but no significant change on HR (378.6 ± 5.40 to 383.6 ± 5.14 bpm; $n = 11$; $t = 1.02$; $P = 0.33$). These microinjections were made at sites where a previous electrical stimulus (30 μA; 50 Hz; 1 ms; 5s trains) had evoked an increase of bladder pressure from 12.2 ± 0.49 to 44.8 ± 1.18 cmH_2O ($n = 18$; $t = 25.4$; $P < 0.0001$) and an increase of BP from 95.6 ± 1.31 to 128.9 ± 2.96 mmHg ($n = 14$; $t = 13.25$; $P < 0.0001$) but failed to elicit any changes in HR (385.4 ± 7.87 to 388.35 ± 8.46 bpm; $n = 14$; $t = 0.68$; $P = 0.5$). Microinjections of saline 0.9% ($n = 10$) made at these sites had no effect on either bladder pressure or pelvic nerve activity.

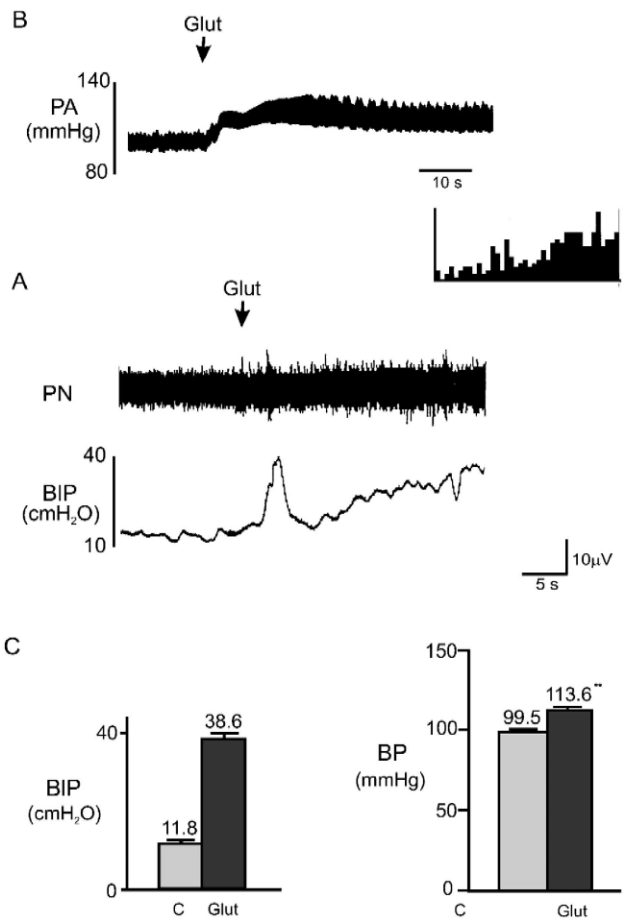

Fig. 2. (A) Effect on arterial blood pressure (BP) of a microinjection of glutamate (Glut) (2 mM) within the locus coeruleus region; (B) Effect of microinjection of glutamate (Glut) on pelvic nerve activity (PN; upper trace) and on bladder pressure (BlP; lower trace) into the same region. The changes on pelvic nerve activity are better seen on the histogram of nerve activity. The initial increase in BlP (together with a burst of nerve activity) is followed by a rise of BlP on which cyclic, but brief, increases in pressure are superimposed (note continued PN activity). (C) Bar graph comparing the baseline levels of BlP and BP with the level of BlP and BP, respectively, after the microinjection of glutamate (Glut)(expressed as mean ± S.E.M.; n(BlP) = 16; $P < 0.0001$; n(BP) = 11; $P < 0.0001$), ** highly significant.

3.1.2. Effect of chemical stimulation with ATP and α,β-meATP

Following stimulation with glutamate a period of 5 min was allowed to elapse before assessing the effect of ATP. At this time baseline values of bladder pressure and arterial blood pressure as well as the resting pattern of nerve activity had been re-established.

Microinjections of ATP at sites where a previous microinjection of glutamate had evoked an increase of bladder pressure and/or an increase in pelvic nerve activity elicited a consistent increase in urinary bladder pressure from 11.3 ± 0.66 to 38.0 ± 1.38 cmH_2O ($n = 11$; $t = 20.43$; $P < 0.0001$) and also a subsequent increase in the rate of contractions (Fig. 3A). Both these effects were preceded by an increase in efferent nerve activity. However, after the injection of ATP, BP fell from 96.3 ± 1.57 to 51.9 ± 7.04 mmHg ($n = 8$; $t = 5.68$; $P < 0.0001$; Figs. 3B and 5A) with no change in HR (378.8 ± 3.98 to 381.9 ± 7.53 bpm; $n = 8$; $t = 0.48$; $P = 0.6$).

Injection of α,β-meATP into the same area of the LC region also evoked an increase in bladder pressure (Fig. 3C) from 11.1 ± 1.16 to 34.7 ± 1.62 cmH_2O ($n = 9$; $t = 20.38$; $P < 0.0001$). Changes in bladder pressure were again preceded by an increase of nerve firing. The microinjection of α,β-meATP into LC consistently evoked a decrease of BP from 101.1 ± 0.92 to 80.8 ± 4.11 mmHg ($n = 7$; $t = 6.39$; $P < 0.0001$; Fig. 3D) but with no change in HR (377.8 ± 8.58 to 382.1 ± 8.01 bpm ($n = 7$; $t = 0.8$; $P = 0.45$). The response to α,β-meATP did not show desensitization as in three animals the responses to repeated injections were identical.

3.1.3. Effect of α,β-meATP after microinjection of suramin

Suramin did not evoke any changes in bladder pressure and arterial blood pressure or in pelvic nerve activity when injected at sites where glutamate, ATP and α,β-meATP had previously evoked changes on bladder pressure ($n = 7$). Microinjection of α,β-meATP 5 min after suramin provoked increases in bladder pressure of 5.0 ± 1.15 cmH_2O which were smaller than those evoked by injections of α,β-meATP alone (21.0 ± 1.38 cmH_2O; $n = 7$; $t = 8.30$; $P < 0.0001$; Fig. 6). No significant changes of BP were observed either after suramin injection or with the microinjection of α,β-meATP after the pretreatment with suramin.

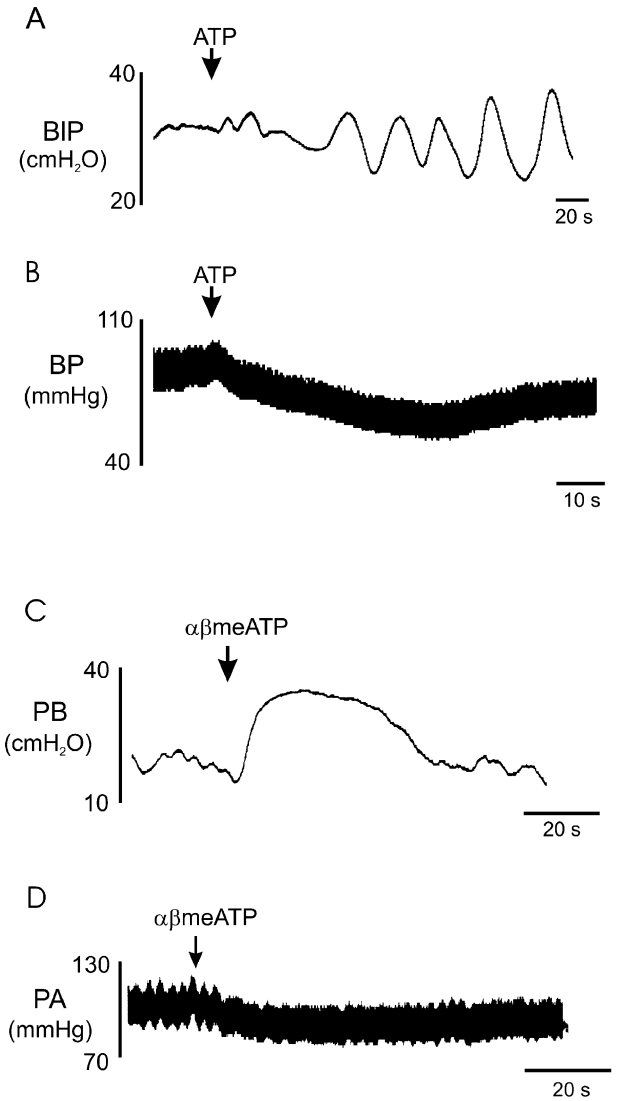

Fig. 3. This figure shows the effect on bladder pressure (BlP)(A) and arterial blood pressure (BP)(B) of the microinjection of ATP (20 mM) into the locus coeruleus region. The application of ATP elicits on BlP a later wave of bladder contractions after the microinjection of the chemical. A tracing of bladder pressure (C) and arterial blood pressure (D) showing the excitatory effect of a microinjection of α,β-meATP (2 mM) within the same region.

3.2. Periaqueductal grey matter

3.2.1. Effect of chemical stimulation with glutamate

Electrical stimulation (30 μA; 50 Hz; 1 ms; 5s trains) within restricted regions of PAG – the ventrolateral, lateral and dorsolateral regions – evoked a rise of bladder pressure from 11.9 ± 0.79 to 46.8 ± 1.47 cmH_2O ($n = 17$; $t = 20.9$; $P < 0.0001$) and an increase on BP from 99.6 ± 1.68 to 143.8 ± 1.91 mmHg ($n = 12$; $t = 15.22$; $P < 0.0001$) without changes in HR (379.2 ± 5.86 to 385.4 ± 6.26 bpm; $n = 12$; $t = 1.11$; $P = 0.29$). This pattern of response was observed also after the microinjection of glutamate. In this situation the increase in bladder pressure was from 12.4 ± 0.59 to 40.2 ± 2.16 cmH_2O ($n = 16$; $t = 12.17$; $P < 0.0001$) and BP rose from 99.7 ± 1.47 to 110.3 ± 1.88 mmHg ($n = 12$; $t = 5.35$; $P < 0.0001$). No significant change of HR was observed (380.4 ± 4.37 to 390.0 ± 4.77 bpm; $n = 12$; $t = 1.88$; $P = 0.08$).

3.2.2. Effect of the microinjection of ATP and its analogue α,β-meATP

Five min after the last microinjection of glutamate, the effect of ATP was assessed. Baseline levels of bladder pressure and nerve activity had been re-established at this time. Microinjection of ATP elicited an increase of urinary bladder pressure from 12.8 ± 0.84 to 46.9 ± 1.87 cmH_2O ($n = 11$; $t = 20.09$; $P < 0.0001$; Fig. 4) and a biphasic change in BP with an initial increase from 96.9 ± 0.79 to 111.3 ± 2.74 mmHg ($n = 8$; $t = 40.6$; $P < 0.005$) followed by a decrease from 111.3 ± 2.74 to 84.4 ± 1.75 mmHg ($n = 8$; $t = 10.44$; $P < 0.0001$; Fig. 5A). The changes in HR during the first phase of BP response were insignificant (375.6 ± 9.23 to 376.3 ± 10.36 bpm; $n = 8$; $t = 0.16$; $P = 0.87$) but during the second phase of the BP response, HR increased significantly from 376.3 ± 10.36

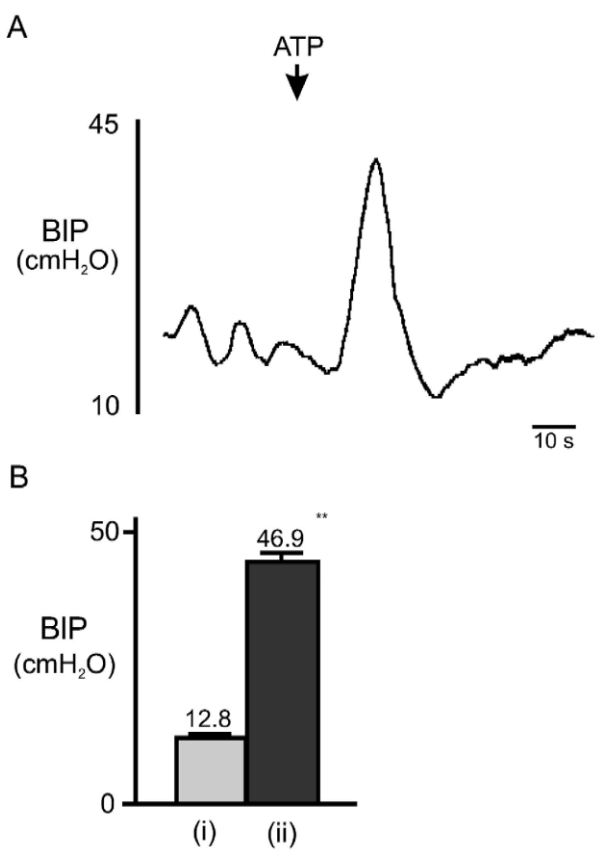

Fig. 4. (A) A tracing of urinary bladder pressure (BlP) showing the effect of a microinjection of ATP (20 mM) within periaqueductal grey matter (PAG). (B) Bar graph illustrating the baseline levels of BlP (light bar) and the changes on BlP after the microinjection of ATP (dark bar) (data expressed as mean ± S.E.M.; $n = 11$; $P < 0.0001$). ** highly significant.

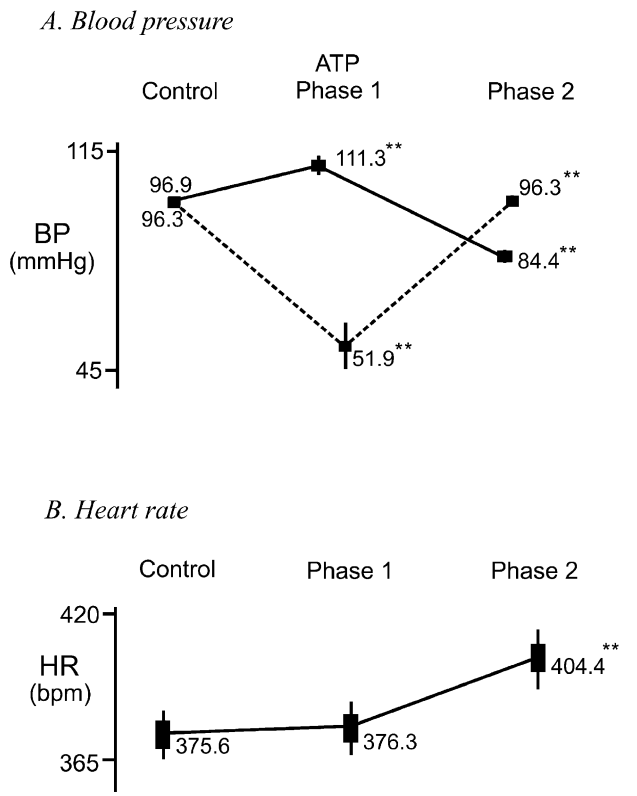

Fig. 5. (A) The effect on arterial blood pressure (BP) of microinjection of ATP on the periaqueductal grey matter (PAG, —) and on the locus coeruleus region (LC, - - -), expressed as mean ± S.E.M. (n(PAG) = 8; n(LC) = 8). (B) The effect on heart rate (HR) of the microinjection of ATP on PAG, expressed as mean ± S.E.M. (n = 8), * significant. * * highly significant.

to 404.4 ± 11.84 bpm (n = 8; t = 4.89; P < 0.005; Fig. 5B) probably reflecting an effective baroreceptor reflex mechanism.

Injection of α,β-meATP into this area of PAG also evoked an increase in bladder pressure from 12.4 ± 0.86 to 30.4 ± 1.41 cmH_2O (n = 10; t = 11.84; P < 0.0001) which was preceded by increase in efferent pelvic nerve activity (Fig. 7). The responses did not show desensitization as the difference between repeated stimulations were not significant. However, the effect of α,β-meATP on BP was inconsistent. Of the seven tests performed with α,β-meATP, five evoked a decrease in BP (of 10.6 ± 1.69) while the other two produced an increase (15 and 25 mmHg). On combining these data, statistical analysis did not show any significant changes in either BP (from 100.7 ± 2.97 to 100.3 ± 8.28 mmHg; n = 7; t = 0.06; P = 0.9) or HR (377.1 ± 4.21 to 375.7 ± 7.44 bpm; n = 7; t = 0.28; P = 0.79).

3.2.3. Effect of microinjection of α,β-meATP after suramin

Following the microinjection of suramin no changes of bladder pressure, BP and HR or pelvic nerve activity were observed. Five min after pre-treatment with suramin, injection of the agonist α,β-meATP at eight sites within PAG evoked small increase in bladder pressure of 2.9 ± 1.46 cmH_2O. This response was smaller than that observed after the injection of α,β-meATP without suramin pre-treatment (18.0 ± 1.68 cmH_2O) indicating that suramin significantly decrease the effect of α,β-meATP (n = 8; t = 9.04; P < 0.0001; Fig. 6). The variable effects of α,β-meATP on BP and HR were not modified by suramin (not tested statistically given the variability of responses).

3.3. Inhibitory sites in PAG and LC region

At three sites within PAG and two sites within LC region, electrical stimulation evoked a small decrease or no change in bladder pressure. Immediately on cessation of stimulation a rise in bladder pressure preceded by a burst

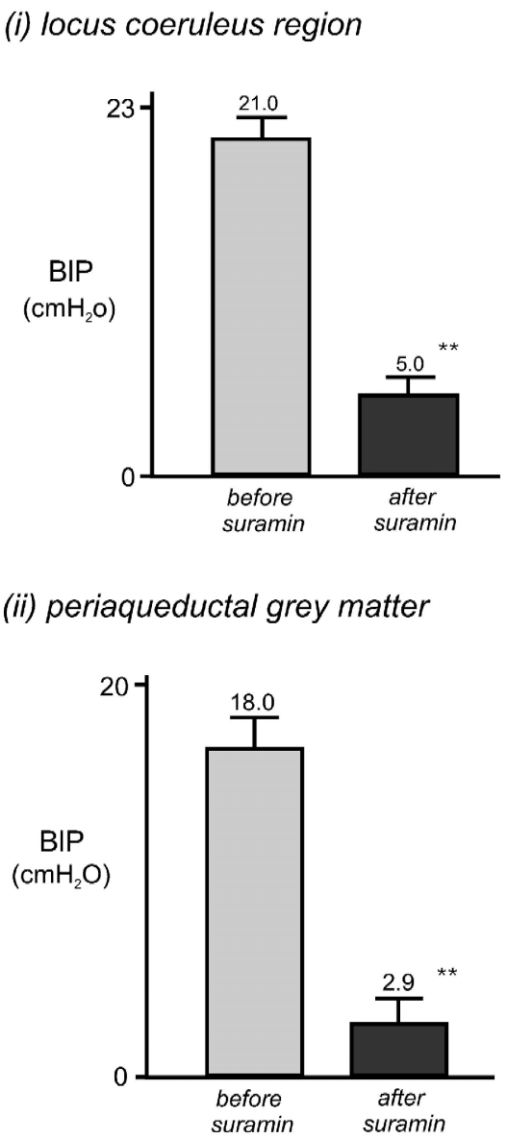

Fig. 6. Changes in bladder pressure (BlP) evoked by the microinjection of α,β-meATP (2 mM) into both LC region (n = 7) and PAG (n = 8) before (light bars) and after suramin (20 mM) pretreatment (dark bars; data expressed as mean ± S.E.M.) * * highly significant.

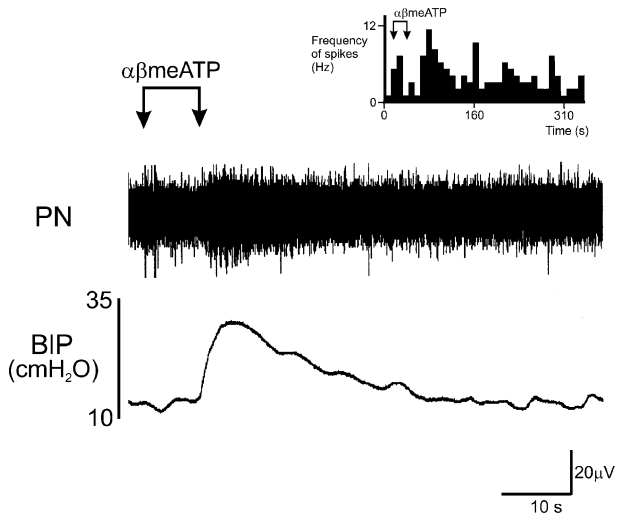

Fig. 7. The effect on pelvic nerve activity (PN; upper trace) and on bladder pressure (BlP; lower trace) of a microinjection of α,β-meATP (2 mM) in PAG. Note that the increase of nerve activity (see the histogram of nerve activity) at the onset of the stimulation precedes the increase in bladder pressure. * * highly significant.

of nerve activity was observed. A similar effect was observed in one case with glutamate stimulation. The sites from which this pattern of response was elicited were shown to be located medially to the sites within PAG and LC region from which excitatory responses were routinely evoked.

3.4. Identification of the stimulation sites

The histological analysis of pontamine sky blue dye staining confirmed that the sites of stimulation which resulted in elevations of urinary bladder pressure and pelvic nerve activity were confined to the locus coeruleus/Barrington nucleus region ($B = -9.00$ to -9.80; $L = 0.70$ to 1.50; $D = 6.00$ to 7.20) and to periaqueductal grey – the ventrolateral, lateral and dorsolateral subdivisions – ($B = -7.00$ to -7.80; $L = 0.40$ to 1.00; $D = 4.80$ to 5.40) (Fig. 8).

4. Discussion

The primary result of this study was the demonstration that electrical and chemical stimulation (with glutamate and ATP) of two areas of the brainstem – one more caudal, the general region of LC, the other more rostral, the PAG – were able to produce changes in urinary bladder pressure, pelvic nerve activity, arterial blood pressure and heart rate. Using electrical stimulation with low intensity to minimise current spread (Bagshaw and Evans, 1986) effective sites were restricted even within these areas. The specific localisation of sites was further shown by the observation that glutamate microinjections at many of these sites also evoked a similar pattern of response, implying that the effect of stimulation was the result of activating cell bodies rather than axons of passage. Both

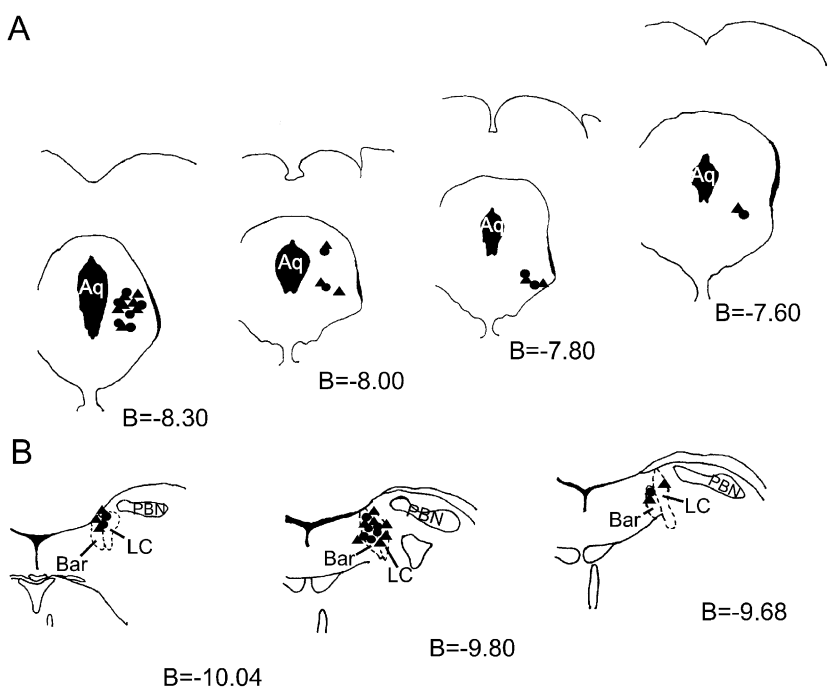

Fig. 8. Representative transverse sections depicting the sites of chemical stimulation with ATP (t) and with α,β-meATP (▲): (A) sites within periaqueductal grey matter (PAG); (B) sites within locus coeruleus region (LC), according to the rat atlas of Paxinos and Watson (1986). Aq., cerebral aqueduct; Bar., Barrington nucleus; PBN., parabracheal nucleus; B., bregma.

electrical and chemical stimulation with glutamate in both areas (LC and PAG) evoked consistent increases in bladder pressure and/or pelvic nerve activity. These data are consistent with those described by other authors using similar approaches to these and other areas of the pons in both anaesthetised cats (Mallory et al., 1991; Chen et al., 1993) and rats (Noto et al., 1989, 1991; Kruse et al., 1990; Von Kügelgen and Starke, 1991). However, our novel observation was that changes in bladder pressure and/or pelvic nerve activity and in the cardiovascular variables could be induced when injecting ATP or its synthetic analogue, α,β-meATP at these sites. This implies that purine receptors may be involved in brainstem-evoked changes in bladder activity and arterial blood pressure.

In the present study, LC and PAG were stimulated, both electrically and with glutamate. Activating LC evoked an increase in pelvic nerve activity which preceded an increase of bladder pressure as previously described by others (for review see Torrens and Morrison, 1987). In PAG also, electrical stimulation at low intensity and microinjection of glutamate evoked a discharge in the pelvic nerve that preceded a rise of bladder pressure. These observations are consistent with anatomical data (Ding et al., 1997) showing the neural connections between sacral spinal cord, LC and PAG, and also with results of previous electrophysiological studies. The neuronal recordings provided evidence for the importance of PAG in the modulation of the micturition reflex (Noto et al., 1991). Furthermore, Ennis et al. (1991) have shown that PAG strongly innervates neurones of the pericoerulear region. Interestingly, in the present study, excitatory responses were highly localised, such that activation of more medial sites in LC and PAG elicited more complex responses. Both electrical stimulation and glutamate microinjection did not elicit either changes of bladder pressure or pelvic nerve activity at these medial sites during administration. However, a rise of bladder pressure preceded by a discharge of the pelvic nerve was observed immediately on cessation of stimulation. This result suggests that these medial sites, when activated, may depress bladder function through interactions with the more lateral sites, and excitation follows a withdrawal of their inhibitory actions. We consistently observed changes in BP when activating sites in both LC and PAG, together with changes in bladder pressure but it was noticeable that there were rarely changes in HR.

The present observations with electrical stimulation and glutamate injection into LC are similar to those described by Chen et al. (1993) who showed in the cat that urinary bladder motility was modified by glutamate activation of neurones located in 'cardiovascular' areas of the brainstem. However, the present observations on changes elicited by glutamate on PAG differ from those of Chen et al. (1993). We observed a consistent increase in both BP and bladder pressure whereas Chen et al. (1993) found that activating the 'pressor areas' of PAG resulted in a decrease in bladder pressure. Whilst the differences do not reflect different experimental protocols, our observations that within PAG there are sites giving differential effects on bladder pressure, may account for the variable relationship between bladder pressure and BP responses. In the present study, stimulation in PAG at sites evoking increases in bladder pressure evoked an increase in BP with no significant changes in HR. The limited changes in cardiovascular activity evoked from PAG differ markedly from studies in the unanaesthetised decerebrate cat where glutamate microinjections elicited cardiovascular features of the defence response with a marked tachycardia (Carrive et al., 1987). The combination of both anaesthetic and paralysing agents used in our study may have contributed to the absence of significant cardiac responses. Nevertheless, the preparation can show changes in HR, as seen in the effects of ATP microinjection (see later).

The metabolic role of intracellular ATP is well established but the work of Holton (1959) showed for the first time that a release of ATP from sensory nerve endings was stimulation-dependent. Subsequently, studies have established the co-storage of ATP with noradrenaline (Von Kügelgen and Starke, 1991) and acetylcholine (Silinsky and Hubbard, 1973). The actions of ATP as a neurotransmitter in the nervous system or as a co-transmitter with either noradrenaline or acetylcholine are well-documented (Burnstock, 1990, 1997). Exogenously applied ATP is generally excitatory via P2 purine receptors although some studies have shown an inhibitory effect usually after a breakdown to adenosine with a decrease of the firing rate of CNS neurones and reduced transmitter release from cholinergic and adrenergic synapses via presynaptic P1 adenosine receptors (Abbrachio, 1997). In a study on rat medial habenula nucleus, Edwards et al. (1992) were able to show that, like glutamate, ATP is a fast excitatory neurotransmitter. Other studies have also suggested that neurones in sensory areas of the brain and in the outer layer of the spinal dorsal horn can be excited by exogenously applied ATP (Jahr and Jessel, 1983; Salt and Hill, 1983; Fyffe and Perl, 1984).

The principal novel observation of the present study was that ATP and its stable analogue α,β-meATP elicited changes on bladder pressure and/or pelvic nerve activity and in BP when injected into both LC and PAG. In fact, ATP microinjections into both PAG and LC consistently evoked a rise of bladder pressure and an increase on pelvic nerve activity. Whether this reflects a direct action of ATP or a modulation of transmitter release remains to be resolved. For LC noradrenaline appears to be a major inhibitory neurotransmitter as it elicits a marked hyperpolarisation of LC neurones (Harms et al., 1992). ATP-evoked excitations could result from a down regulation of tonic noradrenaline release in the LC.

Since ATP is not stable and is rapidly converted by transaminases to adenosine with the possible activation of P1 receptors, α,β-meATP was also used in the present

study. It consistently elicited a significant increase of both bladder pressure and pelvic nerve activity. Desensitising effects of repeated α,β-meATP microinjections have been reported (O'Connor et al., 1991; Edwards et al., 1992; Evans et al., 1992). However, this was not observed in the present study. Harms et al. (1992) have reported that α,β-meATP evoked a depolarising response of LC neurones and did not reveal desensitisation on repeated application in an in vitro study.

From a comparison of the magnitude of the effect of ATP and α,β-meATP on bladder pressure it is conceivable that since the effect of ATP is greater than that of α,β-meATP, ATP-evoked excitation may also involved actions of adenosine. However, further studies are required to address this issue.

Notably the effect of ATP and α,β-meATP on BP was different to that of glutamate. In LC the microinjection of both ATP and α,β-meATP evoked a decrease on BP. The depressor response was more marked to ATP than to α,β-meATP in the doses used in the study, perhaps as a consequence of the fact that α,β-meATP is slowly degraded and acts selectively at a number of P2X receptors (Edwards and Gibbs, 1983; Evans et al., 1992). It is possible that the BP response to ATP reflects the combined action of ATP itself and adenosine produced from its breakdown (Ribeiro, 1991). In PAG, ATP elicited a biphasic response – an increase in BP followed by a fall – whereas α,β-meATP gave highly variable responses. In the majority of sites (5/7) α,β-meATP evoked a rise in BP but at two of seven sites a depressor response was elicited. The later depressor response of ATP was accompanied by a tachycardia. As ATP and α,β-meATP failed to evoke any other changes on HR it is possible that this response reflects a baroreceptor-mediated reflex to the elevation of BP.

In order to examine the specificity of the response evoked by the two agonists, the P2 receptor antagonist, suramin, was also microinjected prior to α,β-meATP. This partially antagonised the action of α,β-meATP on bladder pressure and/or pelvic nerve activity. This partial antagonism may indicate that the dose of suramin was insufficient to block the P2 receptors involved or that a part of the effect of ATP and α,β-meATP is mediated by suramin insensitive receptors. It is clear that whilst purine receptors can be activated by exogenously applied agonists and that they are partially sensitive to the P2 receptor antagonist suramin, these receptors are not tonically active. This conclusion rests on our observations that suramin failed to elicit changes in bladder pressure, pelvic nerve activity and cardiovascular variables when microinjected into both PAG and LC.

The pattern of responses observed in this study for ATP and its analogue, α,β-meATP, could be explained by an action through P2 receptors to elicit fast excitatory actions, implying that ATP may be involved as a primary neurotransmitter or as an excitatory co-transmitter. Since we have observed responses evoked by both ATP and α,β-meATP, and an antagonism, albeit partial in some cases, by suramin, it is likely that the receptors involved are of P2X1, P2X2 or P2X3 subtypes (see Ralevic and Burnstock, 1998). We cannot exclude the involvement of other members of the P2 family of receptors, and clearly other experiments are required to resolve this issue. The absence of obvious desensitisation, which is characteristic of P2X1 and P2X3 receptors (α,β-meATP-sensitive) may also be explained by the experimental protocol used in the present study which involved serial microinjections at single sites in only a very limited number of cases. Also we cannot exclude the possibility that ATP (and α,β-meATP) is acting presynaptically to modulate noradrenaline release (see above) or GABA and glutamate release. Notably whilst both ATP and α,β-meATP elicited increases in bladder pressure when delivered to the PAG, the effects on BP of the two ligands differed, and indeed were inconsistent. Presumably the ligands affect different populations of neurones within PAG – those affecting the parasympathetic outflow to the bladder being distinct from the neurones affecting sympathetic activity to the cardiovascular system.

In conclusion, the present study confirms an important role for both PAG and LC of rat in bladder control. The synaptic mechanisms involved in the inter-relationships between PAG and LC remain to be resolved, as do the local mechanisms within each region. We do, however, have strong indication that P2X receptors are involved in mediating some of these actions.

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