British Journal of Pharmacology (1995) 115, 636-640

Contribution of P_2-purinoceptors to neurogenic contraction of rat urinary bladder smooth muscle

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1 The contribution of P_2-purinoceptors to neurogenic contraction was investigated in rat urinary bladder smooth muscle by measurement of isotonic tension.

2 Contraction of rat urinary bladder smooth muscle induced by electrical stimulation was decreased to $84.19 \pm 3.90\%$ of the control ($n=16$) in the presence of atropine (1 μM), which was further decreased to $38.80 \pm 2.75\%$ of the control ($n=49$) in the presence of both atropine and 10 μM α,β-methylene adenosine 5'-triphosphate (α,β-Me ATP).

3 The contractile response induced by electrical stimulation in the presence of atropine and α,β-Me ATP was decreased to $27.81 \pm 4.07\%$ ($n=23$) and $26.63 \pm 5.01\%$ ($n=15$) of the control, by the addition of 100 μM cibacron blue 3GA and 100 μM suramin, respectively. The application of 100 μM adenosine 5'-*o*-2-thiodiphosphate (ADPβS) in the presence of atropine and α,β-Me ATP decreased the contractile response induced by electrical stimulations to $17.15 \pm 3.71\%$ ($n=15$) of the control.

4 Pretreatment of muscle strips with 100 μM ADPβS significantly reduced the response to either 200 μM α,β-methylene adenosine 5'-diphosphate or 200 μM ADPβS.

5 Uridine 5'-triphosphate (100 μM to 1 mM) concentration-dependently contracted muscle strips, and this contraction was significantly antagonized by desensitization of P_2-receptors with α,β-Me ATP (10 μM), and completely antagonized by pretreatment of muscle strips with both α,β-Me ATP and ADPβS (100 μM).

6 Di(adenosine-5') tetraphosphate (30 and 100 μM) contracted muscle strips, whereas it failed to contract after desensitization of P_2-receptors.

7 It is suggested that about 20% of the neurogenic contraction of rat urinary bladder smooth muscle is mediated via ADPβS-sensitive purinoceptors.

Keywords: P_2-purinoceptors; urinary bladder smooth muscle; α,β-methylene adenosine 5'-triphosphate; adenosine 5'-*o*-2-thiodiphosphate; cibacron blue 3GA; suramin

Introduction

It is well known that there is a variety of receptors in urinary bladder smooth muscles (Hisayama *et al.*, 1988; Iacovou *et al.*, 1990). The existence of P_{2X}-receptors was confirmed in guinea-pig urinary bladder (Burnstock & Kennedy, 1985; Iacovou *et al.*, 1988); in mouse urinary bladder it was reported that P_{2X}- and P_{2Y}-receptors coexist (Boland *et al.*, 1993) and in rat urinary bladder, P_1- and P_{2X}-receptors (Bhat *et al.*, 1989; Nicholls *et al.*, 1992) were shown to exist.

Recently, we reported that a P_2-purinoceptor other than P_{2X}, which mediates contraction, exists in rat urinary bladder (Suzuki & Kokubun, 1994). This type of purinoceptor responds to either adenosine 5'-*o*-2-thiodiphosphate (ADPβS) or α,β-methylene adenosine 5'-diphosphate (α,β-Me ADP) but not to α,β-Me ATP and 2-MeSATP (ADPβS-sensitive purinoceptor), and is, not completely but significantly, antagonized by cibacron blue 3 GA (CB3GA). However, the physiological role of this receptor in rat urinary bladder, i.e. the role of the receptor in neurogenic contraction of the bladder, has not yet been investigated. Therefore, the first aim of this study was to investigate whether ADPβS-sensitive purinoceptors mediate the neurogenic contraction of rat urinary bladder.

Since various P_2-purinoceptors are known to induce contraction of various smooth muscles or increase intracellular Ca^{2+} concentration in various tissues (Fredholm *et al.*, 1994), the second aim of this study was to investigate whether purinoceptors other than P_{2X} and ADPβS-sensitive purinoceptors mediate contraction in rat urinary bladder smooth muscle.

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Methods

Male Wistar strain rats (weighing 200 to 250 g) were anaesthetized with pentobarbitone sodium (40 mg kg^{-1}), and the urinary bladder was rapidly removed. The bladder was transferred into Tyrode solution and then cut into small tissue strips of 7.5×2 mm. The strip was suspended between two platinum-plate electrodes in an organ bath which contained 10 ml of Tyrode solution with the following composition (mM): NaCl 136.9, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 0.5, NaH_2PO_4 0.33, HEPES 5.0 and glucose 5.0 (pH = 7.4). The solution was aerated with O_2 and maintained at 37°C. Responses to drugs as well as electrical stimulations were monitored by measuring isotonic tension under a resting load of 0.5 g, after the strip had been equilibrated for 90 min. After each application of the drug, it was washed out with more than 100 ml of Tyrode solution; the next application of the drug was given at least 30 min later. When the concentration-response curves were performed, each dose was added separately. To desensitize P_{2X} receptors, 10 μM α,β-Me ATP was added 8 min before the experiments, and to desensitize P_{2X} and ADPβS-sensitive purinoceptors simultaneously, both 10 μM α,β-Me ATP and 100 μM ADPβS were added 18 min before the experiments. Guanethidine (5 μM) and atropine (1 μM) were present in experiments shown in Figure 3.

Electrical field stimulation was with pulses of 0.5 ms duration at supramaximal voltage (30-35 V) from a bath drive amplifier SEG-3104 (Nihon Kohden). The strips were stimulated at a frequency of 10 Hz for 1 s in every 2 min. In preliminary experiments, it was established that contractile responses to electrical stimulations were fully abolished by tetrodotoxin, 0.5 μM; such responses were therefore deemed to be wholly neurogenic. Guanethidine (5 μM) was always present in electrical stimulation experiments. When we investigated the

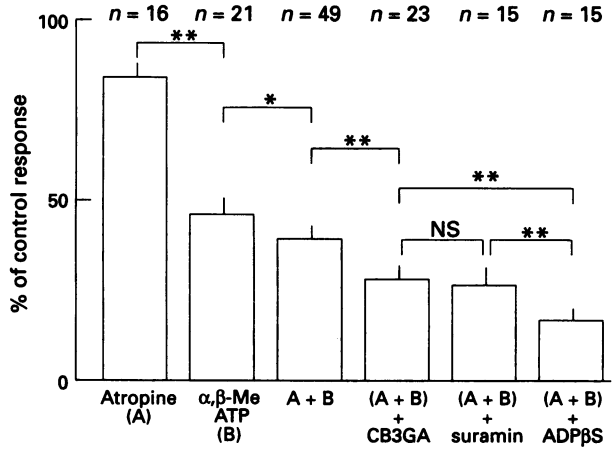

Figure 1 Contractile responses of rat urinary bladder smooth muscle to electrical stimulations in the presence of various agents. Each column represents the mean contractile response and s.e.mean, expressed as a percentage of the control response. Concentrations of agents are: atropine 10^{-6} M; α,β-Me ATP 10^{-5} M; CB3GA 10^{-4} M, suramin 10^{-4} M and ADPβS 10^{-4} M. A+B on the abscissa scale indicate the presence of atropine (10^{-6} M) and α,β-Me ATP (10^{-5} M). CB3GA, suramin and ADPβS were applied in the presence of atropine and α,β-Me ATP. The number of experiments is shown above each column. $*P<0.05$; $**P<0.01$. For abbreviations, see text.

effects of various agents on the contraction induced by electrical stimulation, responses at the steady state were obtained at least 30 min after the application of agents.

Drugs

α,β-Methylene adenosine 5'-diphosphate (α,β-Me ADP), α,β-methylene adenosine 5'-triphosphate (α,β-Me ATP), adenosine 5'-*o*-2-thiodiphosphate (ADPβS), uridine 5'-triphosphate (UTP), uridine 5'-diphosphate (UDP), di(adenosine-5') tetraphosphate (Ap_4A), atropine, guanethidine and cibacron blue 3GA were purchased from Sigma Chemical Company. Acetylcholine chloride (ACh) was purchased from Daiichiseiyaku. Germanin (suramin) was a gift from Bayer. All drugs were prepared freshly before each experiment by dissolving them in Tyrode solution.

Statistical analysis

Results are expressed as mean ± s.e.mean. One-way analysis of variance (one-way anova) was used to test for statistical significance. A probability of 0.05 or less was considered significant.

Results

Inhibition of contractile responses to electrical stimulation by various agents

We investigated the contribution of purinergic regulation in neurogenic contraction of rat urinary bladder (Figure 1). In the presence of atropine (1 μM) the contractile response was decreased to 84.19 ± 3.90% of the control ($n=16$), whereas in the presence of α,β-Me ATP (10 μM) it was decreased to 43.25 ± 3.16% of the control ($n=21$). The inhibitory potency of these drugs was significantly different ($P<0.01$). When we applied both agents simultaneously, the contractile response was decreased to 38.80 ± 2.75% ($n=49$). In the presence of both atropine and α,β-Me ATP, either CB3GA (100 μM) or suramin (100 μM) significantly decreased contraction ($P<0.01$). The contraction observed in the presence of atropine, α,β-Me ATP and CB3GA was 27.81 ± 4.07% ($n=23$), and that in the presence of atropine, α,β-Me ATP and suramin 26.63 ± 5.01% ($n=15$) of the control. They were not significantly different. When we applied ADPβS (100 μM) in the presence of atropine and α,β-Me ATP, the contractile response to electrical stimulation was potently inhibited, being 17.15 ± 3.71% of the control ($n=15$), which was significantly smaller than that observed in any other conditions ($P<0.01$).

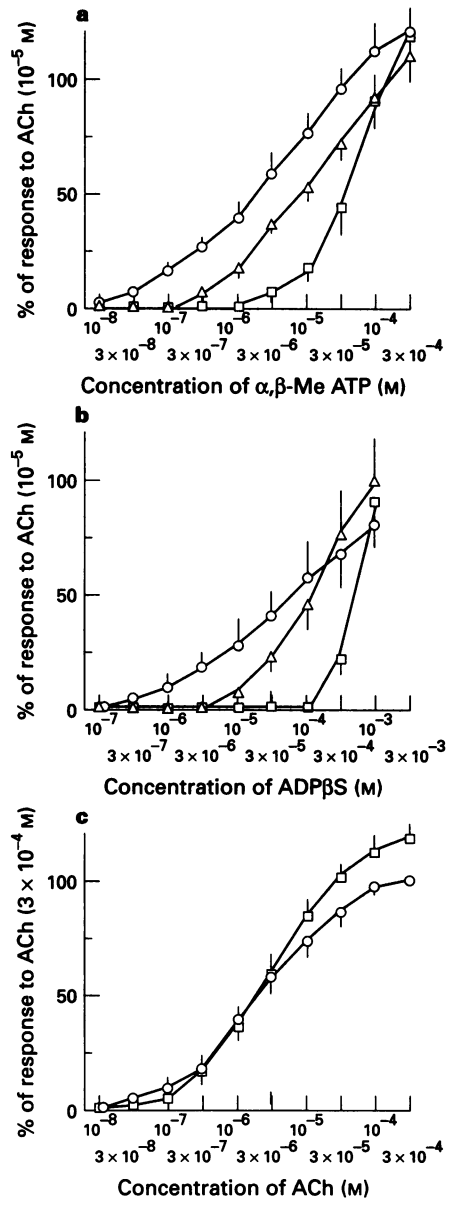

Figure 2 Effects of suramin on concentration-response curves to α,β-Me ATP (a), ADPβS (b) and ACh (c). (a) Responses to α,β-Me ATP in control (○), in the presence of 10^{-5} M suramin (△) and of 10^{-4} M suramin (□) are plotted against the concentration of α,β-Me ATP (M). Each point represents mean ± s.e.mean ($n=5$), expressed as percentage of response to 10^{-5} M ACh. (b) Responses to ADPβS in P_{2X}-desensitized tissues in control (○), in the presence of 10^{-5} M (△) and of 10^{-4} M suramin (□) are plotted against the concentration of ADPβS (M). Each point represents mean ± s.e.mean ($n=6$), expressed as percentage of response to 10^{-5} M ACh. (c) Responses to ACh in control (○) and in the presence of 10^{-4} M suramin (□) are plotted against the concentration of ACh (M). Each point represents mean ± s.e.mean ($n=6$), expressed as a percentage of the response to 3×10^{-4} M ACh. For abbreviations, see text.

Effects of suramin on contractile response to α,β-Me ATP, ADPβS and ACh

Suramin had similar inhibitory effects on the neurogenic contraction of rat urinary bladder to CB3GA in the presence of

α,β-Me ATP and atropine. Since suramin is an antagonist of P_2-purinoceptors (Dunn & Blakely, 1988; Voogd *et al.*, 1993), we investigated the effects of suramin on the concentration-response relations of α,β-Me ATP and ADPβS (Figure 2).

Suramin (10 μM) significantly decreased the contraction induced by α,β-Me ATP at concentrations lower than 100 μM ($P<0.01$), though at 100 and 300 μM, it did not significantly decrease α,β-Me ATP-induced contractions (Figure 2a); 100 μM suramin further decreased contractions. At concentrations of α,β-Me ATP lower than 100 μM, 100 μM suramin was significantly more potent than 10 μM ($P<0.01$ at concentrations lower than 10 μM; $P<0.05$ at 30 μM), though at 100 and 300 μM α,β-Me ATP, 100 μM suramin, like 10 μM, did not significantly inhibit the contraction.

We examined the effect of suramin on ADPβS-induced contraction in P_{2X}-desensitized strips, since ADPβS is known to contract muscle strips not only via ADPβS-sensitive purinoceptors but also via P_{2X}-receptors (Suzuki & Kokubun, 1994) which were relatively antagonized by suramin as shown in Figure 2a (Figure 2b). P_{2X}-receptors were desensitized by pretreatment with α,β-Me ATP (10 μM). Suramin 10 μM significantly inhibited contractions induced by ADPβS at concentrations lower than 100 μM ($P<0.01$ at concentrations lower than 30 μM; $P<0.05$ at 30 μM). At 300 μM and 1 mM ADPβS, suramin slightly augmented contraction rather than inhibited it, though the difference was not significant. Suramin 100 μM completely antagonized contractile effects of ADPβS lower than 300 μM. The mean contractile response to 300 μM ADPβS in the presence of 100 μM suramin was 27.27% of that in the absence of the antagonist, which was significantly smaller ($P<0.01$). At 1 mM ADPβS, 100 μM suramin did not significantly inhibit contraction.

We also examined the effect of suramin (100 μM) on the concentration-response relation of ACh (Figure 2c). Though suramin did not affect the contractile effect of ACh at concentrations lower than 30 μM, it augmented the contraction between 30 and 300 μM ($P<0.05$ at 30 and 100 μM, $P<0.01$ at 300 μM). In the presence of 10 μM suramin, the concentration-response relation of ACh was not significantly affected in four experiments (data not shown).

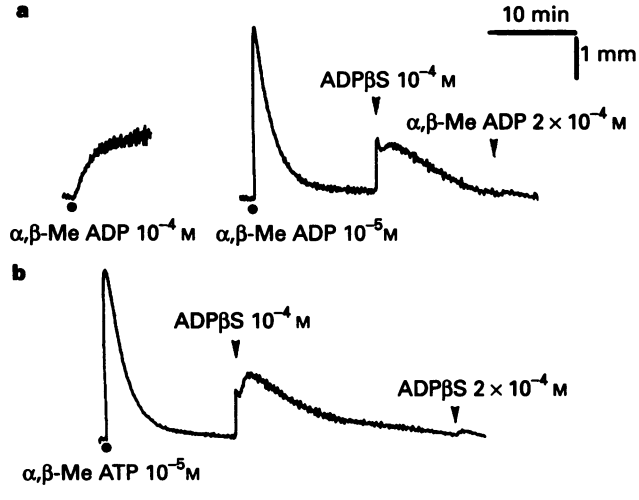

Figure 3 Contraction of rat urinary bladder smooth muscle induced by α,β-Me ADP (a) and ADPβS (b) in the absence and the presence of 10^{-5} M α,β-Me ATP and 10^{-4} M ADPβS. Experiments were done in the presence of atropine (1 μM) and guanethidine (5 μM). At the left side of (a), the control contraction induced by 10^{-4} M α,β-Me ADP is shown. At the right side the contraction induced by 2×10^{-4} M α,β-Me ADP after pretreatment of the muscle strip with 10^{-5} M α,β-Me ATP and 10^{-4} M ADPβS. In (b) the contraction by cumulative application of ADPβS (final concentration $=2\times10^{-4}$ M) in the presence of α,β-Me ATP (10^{-5} M) is shown. All records in this figure were obtained from the same strip. Drugs were applied at (●) or (▼). For abbreviations, see text.

Contraction by ADPβS and α,β-Me ADP in ADPβS-pretreated muscle strips

Since addition of ADPβS in the presence of atropine and α,β-Me ATP most significantly decreased contractile response to electrical stimulation as shown in Figure 1, we examined whether ADPβS desensitized the drug-sensitive purinoceptors. We first examined the contractile response to α,β-Me ADP (100 μM) (Figure 3a) 35 min after the washout of the drug; we treated the muscle strip with both α,β-Me ATP and ADPβS. We applied α,β-Me ATP (10 μM) to desensitize P_{2X}-receptors, and then applied ADPβS (100 μM); 14 min after the application of ADPβS, α,β-Me ADP (200 μM) was applied. The response to 200 μM α,β-Me ADP in the presence of α,β-Me ATP and ADPβS was 5.56% of that to α,β-Me ADP (100 μM) in the absence of these agents in this particular experiment. In 9 experiments the contractile response to α,β-Me ADP in the presence of α,β-Me ATP and ADPβS was 11.31 ± 5.65% of the control. In Figure 3b we examined the contractile response to ADPβS in the muscle strip pretreated with α,β-Me ATP and ADPβS. The experiment was done with the same muscle strip as that used in Figure 3a. We first applied α,β-Me ATP (10 μM), and then applied ADPβS (100 μM). ADPβS (final concentration in the bath: 200 μM) was cumulatively applied 24 min after the first application of the drug. The contractile response to successive applications of ADPβS was 5.75% of

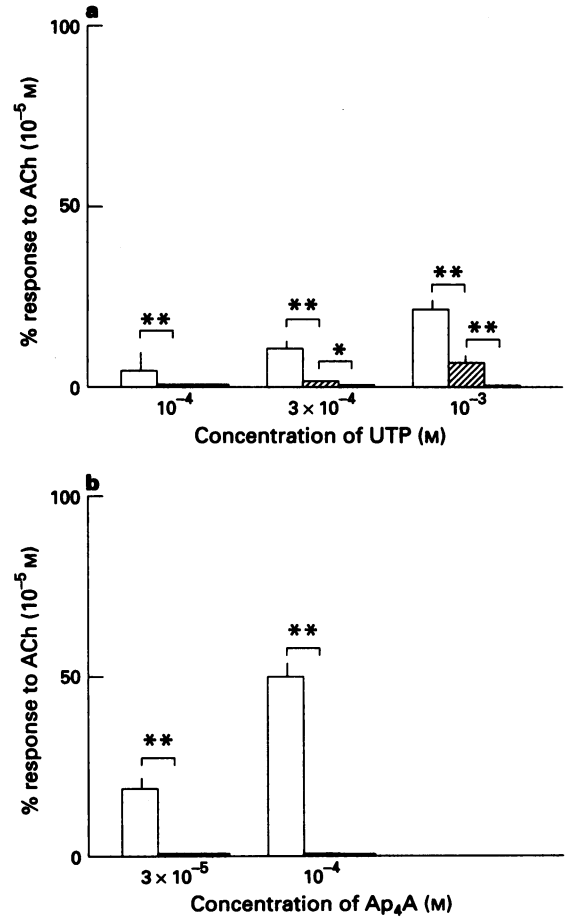

Figure 4 Contractile responses induced by UTP (a) and Ap_4A (b) in the control (open columns), the presence of 10^{-5} M α,β-Me ATP (hatched columns) and the presence of 10^{-5} M α,β-Me ATP and 10^{-4} M ADPβS (solid columns). Each column indicates the mean contractile response and s.e.mean, expressed as a percentage of the response to 10^{-5} M ACh. Abscissae indicate concentrations of drugs (M). The number of experiments in (a) was 13 in all concentrations of UTP, whereas in (b) it was 4 in 3×10^{-5} M and 5 in 10^{-4} M of Ap_4A. $^*P<0.05$, $^{**}P<0.01$. For abbreviations, see text.

that to the first application of the drug. In 9 experiments the response to the second application of ADPβS was 11.17 ± 5.00% of that to the first application.

Effects of UTP, UDP and Ap_4A on urinary bladder smooth muscle

Since ADPβS almost, though not completely, desensitized the drug-sensitive purinoceptors as has been shown in the previous section, we considered the possibility that purinoceptors such as P_{2U} and P_{2D} mediated the neurogenic contraction which still remained in the presence of atropine, α,β-Me ATP and ADPβS. Therefore, we investigated the effects of UTP and Ap_4A on rat urinary bladder smooth muscle. Figure 4a shows the contractile effect of UTP on muscle strips in the control, the presence of α,β-Me ATP (10 μM) and the presence of both α,β-Me ATP (10 μM) and ADPβS (100 μM). UTP concentration-dependently induced contraction; 1.15 ± 0.28% of response to ACh (10 μM) at 30 μM (not included in Figure 4a), 5.00 ± 6.00% at 100 μM, 10.7 ± 1.40% at 300 μM and 22.05 ± 2.09% at 1 mM ($n = 13$). These effects were significantly antagonized by pretreatment with α,β-Me ATP (10 μM). In the presence of α,β-Me ATP UTP did not produce contraction at concentrations lower than 300 μM. At 300 μM and 1 mM UTP the response was 2.05 ± 0.22% and 6.62 ± 1.23% of that to ACh (10 μM), respectively. When we pretreated muscle strips with both α,β-Me ATP (10 μM) and ADPβS (100 μM), UTP at any concentration induced no obvious contraction of muscle strips. We also examined the effects of UDP on urinary bladder smooth muscle. In 11 experiments UDP (< 5 mM) did not induce contraction (data not shown).

We next examined whether Ap_4A induced contraction after desensitization of P_{2X}-receptors as well as ADPβS-sensitive purinoceptors. As shown in Figure 4b, Ap_4A contracted urinary bladder smooth muscle producing 18.08 ± 2.82% of the response to ACh (10 μM) at 30 μM ($n = 4$) and 49.22 ± 3.15% at 100 μM ($n = 5$). In the presence of α,β-Me ATP (10 μM), or α,β-Me ATP (10 μM) plus ADPβS (100 μM), Ap_4A did not contract muscle strips.

Discussion

The contribution of muscarinic receptors to neurogenic contraction was significantly smaller than that of P_{2X}-receptors. About 15% of the neurogenic contraction was via muscarinic receptors, while about 50% of that was via P_{2X}-receptors. After inhibition of both muscarinic receptors and P_{2X}-receptors, about 34% of the control contraction remained. It was interesting to know which receptors mediated the contraction under these conditions.

As previously reported (Suzuki & Kokubun, 1994), ADPβS-sensitive purinoceptors mediating contraction, which respond not to α,β-Me ATP but to either ADPβS or α,β-Me ADP and which are significantly inhibited by CB3GA, exist in rat urinary bladder smooth muscle in addition to P_{2X} receptors. Therefore, in this study we examined whether CB3GA inhibited the neurogenic contraction in the presence of both atropine and α,β-Me ATP. CB3GA significantly inhibited the neurogenic contraction under these conditions, though about 28% of control response still remained.

Suramin, which is a specific P_{2X}-purinoceptor antagonist (Dunn & Blakely, 1988; Voogd *et al.*, 1993), inhibited the neurogenic contraction by a similar extent to CB3GA. We, therefore, examined whether suramin antagonized ADPβS. Though suramin had a more potent inhibitory effect than CB3GA on P_{2X}-mediated contraction, it showed almost the same potency as CB3GA in inhibiting the response to lower concentrations of ADPβS and was less potent than CB3GA in inhibiting the response of ADPβS at higher concentrations (> 100 μM). Suramin did not inhibit but did augment the response to ACh at concentrations higher than 10 μM, though CB3GA did not affect it at all (Suzuki & Kokubun, 1994). These results suggest that CB3GA is a more specific antagonist than suramin at ADPβS-sensitive purinoceptors in rat urinary bladder smooth muscle.

The neurogenic contraction was most potently inhibited by the application of ADPβS in the presence of atropine and α,β-Me ATP. This suggested the possibility that, just as α,β-Me ATP acts as a specific agonist to P_{2X}-receptors as well as a specific antagonist by desensitizing the receptors (Kasakov & Burnstock, 1983), ADPβS also acted as an agonist to ADPβS-sensitive purinoceptors as well as an antagonist by desensitizing them. If this were the case, the contraction inhibited by the application of ADPβS in the presence of atropine and α,β-Me ATP was via ADPβS-sensitive purinoceptors. Indeed, pretreatment of P_{2X}-desensitized muscle strips by ADPβS significantly inhibited the contractions induced by either ADPβS or α,β-Me ADP. These effects were not due to P_{2X}-desensitization by α,β-Me ATP, since we previously found that α,β-Me ATP by itself did not inhibit responses induced by either ADPβS or α,β-Me ADP (Suzuki & Kokubun, 1994). Therefore, the neurogenic contraction inhibited by the application of ADPβS in the presence of atropine and α,β-Me ATP, which was about 20% of the control response, is suggested to be via ADPβS-sensitive purinoceptors.

Which receptor mediated the neurogenic contraction observed in the presence of atropine, α,β-Me ATP and ADPβS, which was about 17% of the control response? While atropine (1 μM) may abolish all cholinergic responses, α,β-Me ATP (10 μM) reduces but does not abolish responses mediated via P_{2X}-purinoceptors in this tissue, as previously reported (Suzuki & Kokubun, 1994). Similarly, ADPβS (100 μM) reduced but did not abolish responses mediated via ADPβS-sensitive purinoceptors. Therefore, the remaining response in the presence of atropine, α,β-Me ATP and ADPβS could be mediated via either P_{2X} and/or ADPβS-sensitive purinoceptors. Alternatively, the remaining response in the presence of atropine, α,β-Me ATP and ADPβS could be mediated by other P_2-purinoceptors, such as P_{2U}, P_{2D}, P_{2Z} or P_{2T}.

Since P_{2U}-receptors respond to UTP and ATP but not to α,β-Me ATP and 2-MeSATP (O'Connor *et al.*, 1991), we examined the effect of UTP on rat urinary bladder smooth muscle. In this preparation, UTP induced contraction, which was significantly inhibited by pretreatment of the muscle with α,β-Me ATP and completely inhibited by pretreatment with both α,β-Me ATP and ADPβS. This indicates that UTP mediated contraction of this tissue was not via P_{2U}-receptors but via P_{2X} and ADPβS-sensitive purinoceptors. We also examined the effect of the P_{2D} agonist, Ap_4A (Hilderman *et al.*, 1991; Castro *et al.*, 1992). The drug induced a stronger contraction than UTP, which was almost completely inhibited by pretreatment with α,β-Me ATP. This indicates that Ap_4A mediated contraction mainly via P_{2X}-receptors. These results suggest that in rat urinary bladder smooth muscle, the neurogenic contraction was mediated by neither P_{2U}- nor P_{2D}-receptors.

P_{2Z}- and P_{2T}-receptors respond to ATP^{4-} and ADP, respectively. The former was reported to exist in macrophages (Steinberg & Silverstein, 1987), mast cells (Dahlqvist & Diamant, 1974) and vas deferens (Fedan *et al.*, 1990), while the latter exists only in platelets (Gordon, 1986). In order to investigate whether P_{2Z}-receptors exist in rat urinary bladder smooth muscle, we would have to examine the dependency of contractile effects of ATP on the concentration of divalent cations, such as extracellular Mg^{2+} concentration, as has been done in guinea-pig vas deferens (Fedan *et al.*, 1990). However, we experienced difficulties in performing quantitative experiments on the effect of ATP, since in rat urinary bladder smooth muscle, adenosine produced by hydrolysis of ATP relaxed muscle strips via A_{2b}-receptors (Suzuki & Kokubun, 1994). Similarly, quantitative experiments on ADP to investigate the existance of P_{2T}-receptors were difficult, since ADP is also hydrolyzed. Therefore, in this study we have not investigated whether the neurogenic contraction in the presence of atropine, α,β-Me ATP and ADPβS is mediated by P_{2Z}- and/or P_{2T}-receptors.

This work was supported by the Ministry of Education, Science and Culture of Japan.

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(Received November 14, 1994
Revised February 17, 1995
Accepted March 1, 1995)