British Journal of Pharmacology (1996) 117, 698-702

Effects of PPADS and suramin on contractions and cytoplasmic Ca^{2+} changes evoked by AP_4A, ATP and α,β-methylene ATP in guinea-pig urinary bladder

Sadaharu Usune, [1]Takeshi Katsuragi & Tatsuo Furukawa

Research Laboratory of Biodynamics, and Department of Pharmacology, School of Medicine, Fukuoka University, Fukuoka 814-80, Japan

1 The contraction and intracellular Ca^{2+} change evoked by diadenosine tetraphosphate (AP_4A) were studied in the outer longitudinal muscle of the guinea-pig urinary bladder and compared with those evoked by ATP and α,β-methylene ATP (a P_2-purinoceptor agonist).

2 AP_4A, ATP and α,β-methylene ATP produced concentration-dependent transient contractions. These contractions were inhibited by PPADS (pyridoralphosphate-6-azophenyl- 2'-4'-disulphonic acid), 0.3-30 μM, a P_{2X}-purinoceptor antagonist, and suramin, 1-300 μM, a P_2-purinoceptor antagonist in a concentration-dependent manner. From Schild plot analysis, the apparent pA_2 values for PPADS for contractions evoked by AP_4A, ATP and α,β-methylene ATP were 6.86, 6.56, 6.74, and those for suramin were 6.01, 4.59 and 5.12, respectively; the Schild slopes for PPADS were 1.07, 1.14 and 1.06, and, those for suramin 0.75, 1.05 and 1.16, respectively.

3 AP_4A (10 μM) and ATP (100 μM) failed to elicit any contraction of the tissue after a desensitization produced by repeated application of α,β-methylene ATP (1 μM).

4 In fluorescence experiments with fura-2, the increases in $[Ca^{2+}]_i$ and contraction evoked by AP_4A were suppressed by suramin and nifedipine, an L-type Ca^{2+} channel blocker.

5 These findings suggest that P_{2X}-purinoceptors, which are more sensitive to PPADS than suramin, exist on the outer longitudinal muscles of guinea-pig urinary bladder, and that the AP_4A-evoked contraction results from Ca^{2+} influx.

Keywords: P_{2X}-purinoceptor; AP_4A; ATP; α,β-methylene ATP; PPADS; suramin; cytoplasmic Ca^{2+} concentration; nifedipine; guinea-pig urinary bladder

Introduction

Purine nucleosides and nucleotides, such as adenosine and ATP, are well known to exert potent extracellular actions on a variety of tissues. Diadenosine polyphosphates, AP_4A, AP_5A and AP_6A, are stored at high concentrations in bovine adrenal chromaffin cells (Rodriguez del Castillo *et al.*, 1988; Pintor *et al.*, 1992b) and rat brain synaptic terminals (Pintor *et al.*, 1992a). It has been reported that AP_4A, AP_5A and AP_6A, which are present in rat brain synaptic terminals, may have a possible role as neurotransmitters. AP_4A, compared to AP_5A and AP_6A, has high affinity binding sites in rat brain and appears to be released from the nerve terminals in a Ca^{2+}-dependent manner (Pintor & Miras-Portugal, 1993). In addition, AP_4A and AP_5A are coreleased with ATP and catecholamines by carbachol from bovine adrenal medulla and chromaffin cells (Pintor *et al.*, 1991). AP_4A modulates catecholamine release from chromaffin cells (Castro *et al.*, 1990). Ecto-phosphodiesterase activity is recognized in the plasma membranes of chromaffin cells, but is much lower than ecto-ATPase activity, so the lifetimes of AP_4A and AP_5A are thought to be much longer than that of ATP (Miras-Portugal *et al.*, 1990). Also, it has been demonstrated that AP_4A exerts vasoactive effects in peripheral arteries via purinoceptors (Busse *et al.*, 1988).

In the present study, we have evaluated the pharmacological characteristics of contractile responses to AP_4A compared with those to ATP and α,β-methylene ATP, a potent P_{2X}- and weak P_{2Y}-purinoceptor agonist (Fredholm *et al.*, 1994), using PPADS (pyridoralphosphate-6-azophenyl-2'-4'-disulphonic acid) and suramin, P_2-purinoceptor antagonists, in the outer longitudinal muscle of guinea-pig urinary bladder. In addition, a fluorescence study measuring changes of cytoplasmic Ca^{2+} concentration, $[Ca^{2+}]_i$, was also carried out using fura-2, a cytoplasmic Ca^{2+} indicator.

[1] Author for correspondence.

Methods

Measurements of tension developments

Male guinea-pigs, 250-350 g, were killed by cervical dislocation and the urinary bladder rapidly removed. The thin outer layer longitudinal muscle preparation, about 1 mm in width and 1 mm in length, of urinary bladder was made by completely removing the epithelium, lamina propria and inner longitudinal muscle and then by removal of much of the middle circular muscle from the urinary bladder by the technique previously described (Usune *et al.*, 1986). The segment was suspended in a perfusing bath, about 0.1 ml, with a modified Krebs solution at 32°C during the experiment in order to inhibit generation of the spontaneous contraction. The Krebs solution contained (mM): NaCl 121.4, KCl 5.9, $CaCl_2$ 2.5, $MgCl_2$ 1.2, $NaHCO_3$ 15.5 and glucose 11.5. The solution was kept at pH 7.4 by bubbling with 95% O_2 and 5% CO_2. After loading the segment with a tension of 1.5 mN, contractions were isometrically recorded as previously described (Usune *et al.*, 1986). Nifedipine, 100 μM, was dissolved in ethanol, 50%, before dilution in distilled water. Final concentration of the solvent was limited to 0.05%. The solvent at this concentration did not significantly affect contractions. Other agents were dissolved in distilled water. AP_4A, ATP and

α,β-methylene ATP were perfused for 0.5–1 min at intervals of 20 min. pA_2 and slope values were determined from Schild plots using the Schild equation described previously (Arunlakshana & Schild, 1959).

Measurements of changes of $[Ca^{2+}]_i$ and tension development

The outer longitudinal muscle segment, about 2 mm in width and 7 mm in length, of urinary bladder was fixed on a silicon board, with one end of the tissue, about 1 mm in length, remaining unfixed for measurement of force development. The tissue was incubated in Krebs solution containing 5 μM fura-2/AM using the technique previously described (Usune *et al.*, 1989), and then rinsed. Thereafter, the segment was suspended in a perfusing bath, about 0.7 ml, and perfused at 3.6 ml min^{-1}. Contractions were recorded isometrically as previously described (Usune *et al.*, 1989).

Fluorescence signals were measured by a double beam fluorimeter (Japan Spectroscopic, CAM-220, Tokyo, Japan). The muscle strips, which were loaded with fura-2, were illuminated alternately, 400 Hz, at two excitation wavelengths, 340±10 nm and 380±10 nm (emission wavelength, 510±0.5 nm). The increase in the relative $[Ca^{2+}]_i$ of the muscle strips which were successfully loaded with fura-2 was measured from an increase of the fluorescence ratio. The changes in $[Ca^{2+}]_i$ were calculated from the peak height of the signal by use of a calibration curve between fura-2 and Ca^{2+} in distilled water.

Drugs

The drugs used were AP_4A (a kind gift from Fujilevio, Tokyo, Japan); suramin (a kind gift from Bayer, Leverkusen, Germany); PPADS (RBI, Natick, U.S.A.); ATP (Boehringer, Mannheim, Germany); α,β-methylene ATP and nifedipine (Sigma, St. Louis, MO, U.S.A.) and Fura-2/AM (Dojindo, Kumamoto, Japan).

Statistical analysis

Statistical significances were assessed by Student's *t* test for values in two samples and by Dunnett's test for values in multiple samples. A value of less than 0.05 was considered as a significant difference.

Results

Contractile responses to AP_4A, ATP and α,β-methylene ATP

AP_4A, ATP and α,β-methylene ATP (1–1000 μM) caused transient contractions in the longitudinal muscle preparation (Figure 1). The concentration-response curves for AP_4A, ATP and α,β-methylene ATP are shown in Figure 1b, indicating that α,β-methylene ATP is the most potent agonist.

Schild plots of PPADS and suramin for contractile responses to AP_4A, ATP and α,β-methylene ATP

The contractions evoked by AP_4A, ATP and α,β-methylene ATP were concentration-dependently suppressed following exposure to PPADS, 0.3–30 μM, and suramin, 1–300 μM for up to 70 min. There was almost no change between the inhibitory effects of the antagonists at different incubation periods, e.g. 10 and 30 min. Concentration-response curves for AP_4A, ATP and α,β-methylene ATP were constructed in the presence and absence of PPADS or suramin (Figures 2 and 3). From these curves, Schild plots were made by plotting log (dose-ratio−1) for AP_4A, ATP and α,β-methylene ATP against log molar concentrations of PPADS or suramin (Figure 4). The apparent pA_2 values of PPADS as an antagonist against AP_4A, ATP and α,β-methylene ATP were 6.86, 6.56 and 6.74, respectively. Similarly, those of suramin were 6.01, 4.59 and 5.12. In addition, the apparent slope values (correlation coefficient, *r*) for PPADS against AP_4A, ATP and α,β-

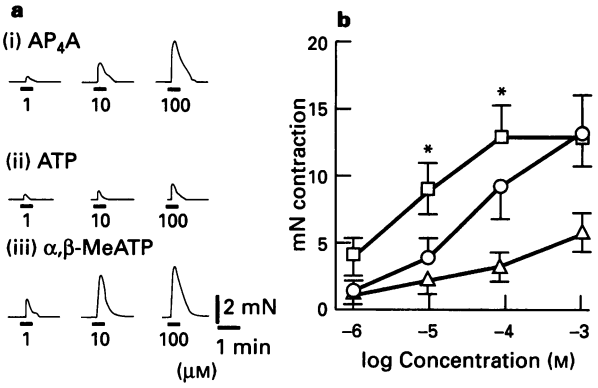

Figure 1 Contractile responses to AP_4A, ATP and α,β-methylene ATP. (a) Typical recording of responses to AP_4A (i), ATP (ii) and α,β-methylene ATP (α,β-MeATP), (iii), in same longitudinal muscle segment of guinea-pig urinary bladder. (b) Concentration-response curves of AP_4A (○), ATP (△) and α,β-methylene ATP (□). Values are expressed as mN (mean±s.e.mean, $n=5$) of AP_4A-, ATP- and α,β-methylene ATP-induced contractions. $^*P<0.05$ compared with values of ATP-induced contraction at corresponding concentration.

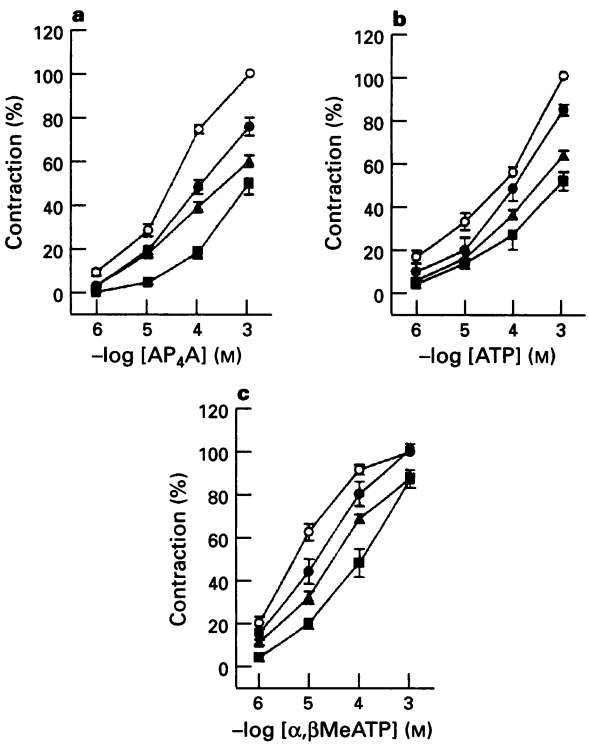

Figure 2 Concentration-response curves of AP_4A (a), ATP (b) and α,β-methylene ATP (c) in the presence and absence of PPADS. Responses in the absence (○) and the presence of 0.3 (●), 1 (▲), 3 μM (■) PPADS. The experiment was carried out by repeated applications (every 20 min) of an agonist at concentrations ranging from 1 μM to 1 mM in the presence of PPADS (0.3, 1 or 3 μM). PPADS was first introduced into the perfusate 10 min before 1 μM agonist. Therefore, agonist at the highest concentration was added 70 min after exposure to the agonist. Values are expressed as percentage change (mean) from each maximum amplitude of AP_4A-, ATP- and α,β-methylene ATP-induced contractions (100%) in the absence of PPADS. Each point indicates mean±s.e.mean from 4-5 experiments.

methylene ATP were 1.07 ($r=0.95$), 1.14 ($r=0.93$) and 1.06 ($r=0.93$), and those of suramin were 0.75 ($r=0.82$), 1.05 ($r=0.97$) and 1.16 ($r=0.95$), respectively.

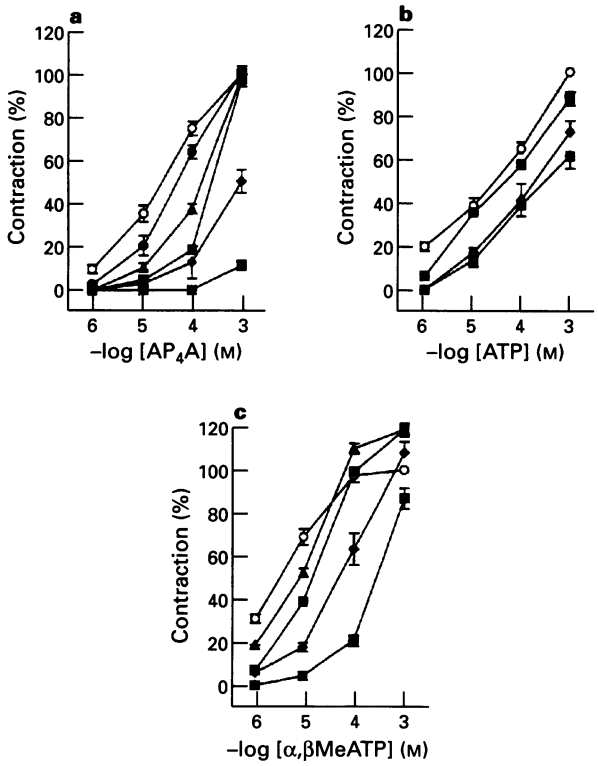

Figure 3 Concentration-response curves of AP_4A (a), ATP (b) and α,β-methylene ATP (c) in the presence and absence of suramin. Responses in the absence (○) and the presence of 1 (●), 10 (▲), 30 (■), 100 (◆) and 300 μM (▼) suramin. The experiment with suramin was carried out following the same procedure as that used for PPADS as described in Figure 2. Values are expressed as a percentage change (mean) from each maximum amplitude of AP_4A-, ATP- and α,β-methylene ATP-induced contractions (100%) in the absence of suramin. Each point indicates mean ± s.e.mean from 4-5 experiments.

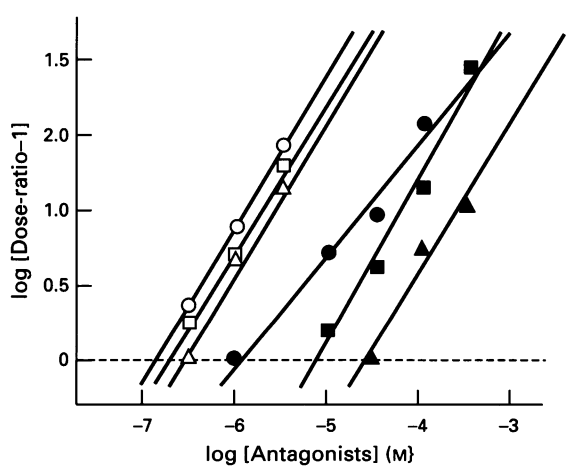

Figure 4 Schild plots of inhibition by PPADS or suramin of AP_4A-, ATP- and α,β-methylene ATP-evoked contractions. Concentration-ratio curves of AP_4A (○ and ●), ATP (△ and ▲) and α,β-methylene ATP (□ and ■) denote ratio of 50% responses induced by agonists in the presence and absence of PPADS (open symbols) or suramin (closed symbols) at various concentrations. PPADS and suramin were added to the solution 10 min before application of AP_4A, ATP and α,β-methylene ATP. Each point indicates mean values from 4-5 experiments.

Cross-desensitization between AP_4A or ATP and α,β-methylene ATP

As shown in Figure 1b, the concentrations of AP_4A and α,β-methylene ATP required to produce contractions of similar amplitude as evoked by ATP, 100 μM, were approximately 10 and 1 μM ($n=10$), respectively. Hence, AP_4A, ATP and α,β-methylene ATP were used at 10, 100 and 1 μM, respectively, in the following experiment. Contractions induced by α,β-methylene ATP gradually decreased during repeated application (twenty times) (Figures 5a(ii) and 5b(ii)). Contractions evoked by AP_4A and ATP were similarly reduced by the same procedure (data not shown). After repeated application of α,β-methylene ATP, the contractions induced by AP_4A and ATP were abolished (Figure 5). The AP_4A- and ATP-induced contractions recovered to their original levels after washout of α,β-methylene ATP.

Effects of suramin and nifedipine on change of $[Ca^{2+}]_i$ and contraction induced by AP_4A

The effects of suramin and nifedipine, an L-type Ca^{2+} channel blocker, on the rise of $[Ca^{2+}]_i$ and contraction evoked by AP_4A are shown in Figure 6. AP_4A, 20 μM, caused an increase in $[Ca^{2+}]_i$, coupled with the contraction (Figure 6a(i)). Both the increases in $[Ca^{2+}]_i$ and contraction were reduced after exposure to suramin, 30 μM, (Figure 6a(ii)). Following administration of nifedipine, 100 nM, the increases in $[Ca^{2+}]_i$ and contraction were also reduced (Figure 6b).

Discussion

It is well-documented that activation of P_{2X} purinoceptors mediates contraction, not relaxation, of various smooth muscles (Fredholm *et al.*, 1994). In the present study, AP_4A, ATP and α,β-methylene ATP produced transient contractions of the outer longitudinal muscle from guinea-pig urinary bladder.

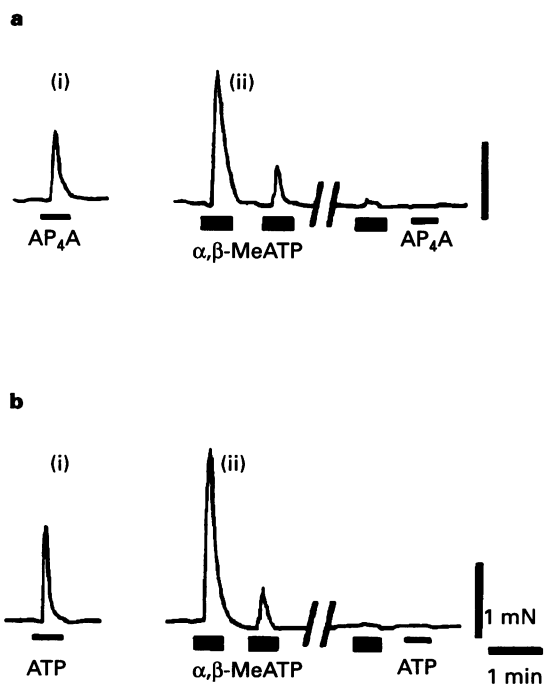

Figure 5 Typical recordings of contractions showing interactions between α,β-methylene ATP and (a) AP_4A or (b) ATP. (i) Control responses to AP_4A, 10 μM, and ATP, 100 μM. (ii) Responses to AP_4A and ATP shown in the same tissues after repeated applications of α,β-methylene ATP (α,β-MeATP), 1 μM. α,β-methylene ATP was added twenty times at 30 s intervals.

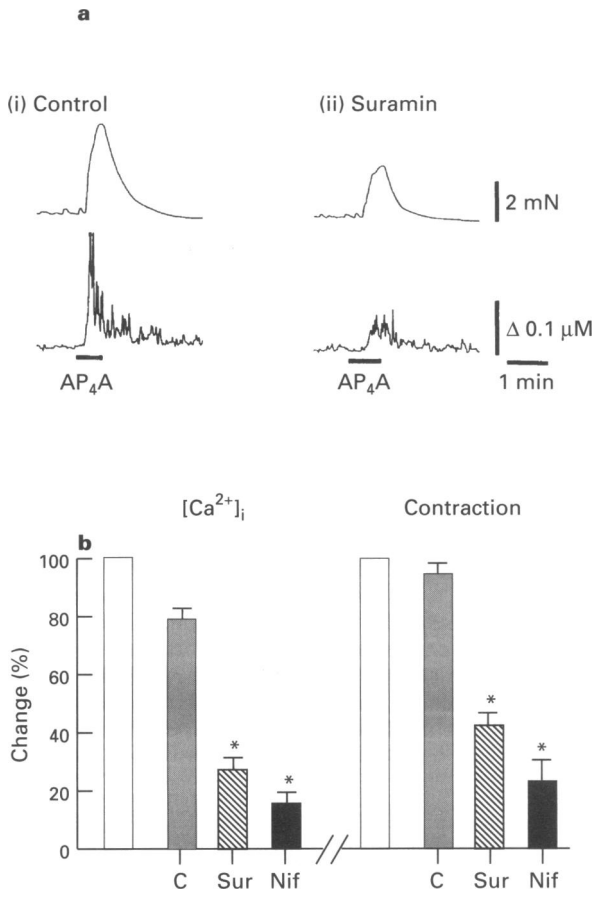

Figure 6 Effects of suramin and nifedipine on the increase in cytoplasmic Ca^{2+} concentration and contraction induced by AP_4A. (a) Contraction (upper traces) and cytoplasmic Ca^{2+} concentrations ($[Ca^{2+}]_i$) (lower traces) in the same preparation. Responses to AP_4A, 20 μM, in the absence (i) and presence (ii) of suramin, 30 μM. (b) Statistical analysis for $[Ca^{2+}]_i$ and contraction evoked by AP_4A. Open columns: each response (100%) to first application of AP_4A. Stippled columns: control response to second application of AP_4A (C). Hatched and solid columns: second responses in the presence of suramin (Sur), 30 μM, and nifedipine (Nif), 100 nM, respectively. Second application: AP_4A was added to the solution 20 min after first application of the agonist. Suramin and nifedipine were added to the solution 10 min before second application of the agonist. Values in all columns are expressed as percentage change from the responses (100%) to first appliction of AP_4A. Each point indicates mean ± s.e.mean ($n=5$). $^*P<0.001$ compared with responses induced by second application of AP_4A in the absence of suramin or nifedipine.

The AP_4A-, ATP- and α,β-methylene ATP-induced contractions were concentration-dependently suppressed by PPADS, a selective P_{2X}-purinoceptor antagonist, as demonstrated in the rabbit vasa deferentia (Lambrecht *et al.*, 1992), urinary bladder (Ziganshin *et al.*, 1993) and blood vessels (Ziganshin *et al.*, 1994) and guinea-pig vas deferens (McLaren *et al.*, 1994). In addition, these agonist-evoked contractions were also inhibited by suramin, an antagonist of P_2-purinoceptors as reported previously for the vas deferens of mouse (Dunn & Blakely, 1988) and urinary bladder (Hoyle *et al.*, 1990), ileum and vas deferens (Katsuragi *et al.*, 1991; Bailey & Hourani, 1995) of guinea-pig. The slopes from the Schild plot analysis with PPADS indicate that PPADS produces a competitive antagonism of AP_4A, ATP and α,β-methylene ATP. However, the slopes from responses to AP_4A in the presence of PPADS and suramin were 1.07 and 0.75 respectively. The pA_2 values obtained from the Schild plot analysis with PPADS were 6.86, 6.56 and 6.74 to AP_4A, ATP and α,β-methylene ATP, respectively. On the other hand, suramin produced more potent antagonism of the response to AP_4A ($pA_2=6.01$) than to ATP ($pA_2=4.59$) and α,β-methylene ATP ($pA_2=5.12$), presumably, via P_{2X}-purinoceptors. Such discrepant findings between PPADS and suramin in agonist-induced responses may be explained by inhibition of ecto-ATPase by suramin in attenuating the breakdown of ATP in guinea-pig urinary bladder (Hourani & Chown, 1989) and rabbit ear artery (Crack *et al.*, 1994) although further investigation is needed for understanding this in detail.

It has been proposed that the ATP-induced contraction is blocked following desensitization of P_{2X}-purinoceptors by α,β-methylene ATP in urinary bladder (Kasakov & Burnstock, 1983; Katsuragi *et al.*, 1986), vas deferens of guinea-pig (Meldrum *et al.*, 1983; Mackenzie *et al.*, 1988) and mouse (von Kügelgen *et al.*, 1990) and human urinary bladder (Hoyle *et al.*, 1989). In the present study, α,β-methylene ATP was more potent than AP_4A and ATP in causing contractions. The contractions induced by AP_4A, ATP and α,β-methylene ATP were abolished after repeated application of each agonist, namely, via autodesensitization. The contractions induced by AP_4A and ATP were abolished after repeated applications of α,β-methylene ATP at low concentration. The data further support the idea that contractions induced by AP_4A, ATP and α,β-methylene ATP are evoked via stimulation of common P_{2X}-purinoceptors.

In the fluorescence study with fura-2, AP_4A evoked an increase in $[Ca^{2+}]_i$, coupled with the contraction. The increases in $[Ca^{2+}]_i$ were inhibited by suramin and nifedipine. It has been reported that increases in $[Ca^{2+}]_i$ and contractions induced by ATP are suppressed by nifedipine in the outer longitudinal muscle of urinary bladder (Katsuragi *et al.*, 1990b) and ileum (Katsuragi *et al.*, 1990a) of guinea-pig. From these data, the AP_4A-induced contraction may be evoked by an increase in $[Ca^{2+}]_i$ following Ca^{2+} influx, probably, via activation of cation-channels.

In conclusion, these results indicate that contractions evoked by AP_4A, ATP and α,β-methylene ATP via activation of P_{2X}-purinoceptors are antagonized by lower concentrations of PPADS than by suramin in the outer longitudinal muscle of guinea-pig urinary bladder, and that AP_4A, like ATP and α,β-methylene ATP, increases Ca^{2+} influx.

This work was partly supported by Grant-in-Aid for Scientific Research (02670102, T.K.) from the Ministry of Education, Sciences and Culture of Japan. The authors are grateful to Fujilevio Ltd and Bayer Ltd for kindly providing AP_4A and suramin, respectively.

References

ARUNLAKSHANA, O. & SCHILD, H.O. (1959). Some quantitative uses of drug antagonists. *Br. J. Pharmacol. Chemother.*, **14**, 48–58.

BAILEY, S.J. & HOURANI, S.M.O. (1995). Effects of suramin on contractions of the guinea-pig vas deferens induced by analogues of adenosine 5'-triphosphate. *Br. J. Pharmacol.*, **114**, 1125–1132.

BUSSE, R., OGILVIE, A. & POHL, U. (1988). Vasomotor activity of diadenosine triphosphate and diadenosine tetraphosphate in isolated arteries. *Am. J. Physiol.*, **254**, H828–H832.

CASTRO, E., TORRES, M., MIRAS-PORTUGAL, M.T. & GONZALEZ, M.P. (1990). Effect of diadenosine polyphosphates on catecholamine secretion from isolated chromaffin cells. *Br. J. Pharmacol.*, **100**, 360–364.

CRACK, B.E. BEUKERS, M.W., MCKECHNIE, K.C.W., IJZERMAN, A.P. & LEFF, P. (1994). Pharmacological analysis of ecto-ATPase inhibition: evidence for combined enzyme inhibition and receptor antagonism in P_{2X}-purinoceptor ligands. *Br. J. Pharmacol.*, **113**, 1432–1438.

DUNN, P.M. & BLAKELEY, A.G.H. (1988). Suramin: a reversible P_2-purinoceptor antagonist in the mouse vas deferens. *Br. J. Pharmacol.*, **93**, 243–245.

FREDHOLM, B.B., ABBRACCHIO, M.P., BURNSTOCK, G., DALY, J.W., HARDEN, T.K., JACOBSON, K.A., LEFF, P. & WILLIAMS, M. (1994). Nomenclature and classification of purinoceptors. *Pharmacol. Rev.*, **46**, 143–156.

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

HOYLE, C.H.V., KNIGHT, G.E. & BURNSTOCK, G. (1990). Suramin antagonizes responses to P_2-purinoceptor agonists and purinergic nerve stimulation in the guinea-pig urinary bladder and taenia coli. *Br. J. Pharmacol.*, **99**, 617–621.

HOURANI, S.M.O. & CHOWN, J.A. (1989). The effects of some possible inhibitors of ectonucleotidases on the breakdown and pharmacological effects of ATP in the guinea-pig urinary bladder. *Gen. Pharmacol.*, **20**, 413–416.

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

KATSURAGI, T., KURATOMI, L. & FURUKAWA, T. (1986). Clonidine-evoked P_1-purinoceptor antagonism of contraction of guinea pig urinary bladder. *Eur. J. Pharmacol.*, **121**, 119–122.

KATSURAGI, T., TOKUNAGA, T., OGAWA, S., SOEJIMA, O., SATO, C. & FURUKAWA, T. (1991). Existence of ATP-evoked ATP release system in smooth muscles. *J. Pharmacol. Exp. Ther.*, **259**, 513–518.

KATSURAGI, T., TOKUNAGA, T., USUNE, S. & FURUKAWA, T. (1990a). A possible coupling of postjunctional ATP release and transmitters' receptor stimulation in smooth muscles. *Life Sci.*, **46**, 1301–1307.

KATSURAGI, T., USUNE, S. & FURUKAWA, T. (1990b). Antagonism by nifedipine of contraction and Ca^{2+}-influx evoked by ATP in guinea-pig urinary bladder. *Br. J. Pharmacol.*, **100**, 370–374.

LAMBRECHT, G., FRIEBE, T., GRIMM, U., WINDSCHEIF, U., BUNGARDT, E., HILDEBRANDT, C., BÄUMERT, H.G., PATZ-KÜMBEL, G. & MUTSCHLER, E. (1992). PPADS, a novel functionally selective antagonist of P_2 purinoceptor-mediated responses. *Eur. J. Pharmacol.*, **217**, 217–219.

MACKENZIE, I., KIRKPATRICK, K.A. & BURNSTOCK, G. (1988). Comparative study of the actions of AP_5A and α,β-methylene ATP on nonadrenergic, noncholinergic neurogenic excitation in the guinea-pig vas deferens. *Br. J. Pharmacol.*, **94**, 699–706.

MCLAREN, G.J., LAMBRECHT, G., MUTSCHLER, E., BÄUMERT, H.G., SNEDDON, P. & BURNSTOCK, G. (1994). Investigation of the action of PPADS, a novel P_{2X}-purinoceptor antagonist, in the guinea-pig isolated vas deferens. *Br. J. Pharmacol.*, **111**, 913–917.

MELDRUM, L.A. & BURNSTOCK, G. (1983). Evidence that ATP acts as a cotransmitter with noradrenaline in the sympathetic nerves supplying the guinea-pig vas deferens. *Eur. J. Pharmacol.*, **92**, 161–163.

MIRAS-PORTUGAL, M.T., PINTOR, J., ROTLLAN, P. & TORRES, M. (1990). Characterization of ectonucleotidases in chromaffin cells. *Ann. N.Y. Acad. Sci.*, **603**, 523–526.

PINTOR, J., DIAZ-REY, M.A., TORRES, M. & MIRAS-PORTUGAL, M.T. (1992a). Presence of diadenosine polyphosphates-Ap_4A and -Ap_5A in rat brain synaptic terminals. Ca^{2+}-dependent release evoked by 4-aminopyridine and veratridine. *Neurosci. Lett.*, **136**, 141–144.

PINTOR, J. & MIRAS-PORTUGAL, M.T. (1993). Diadenosine polyphosphates (Ap_xA) as new neurotransmitters. *Drug Dev. Res.*, **28**, 259–262.

PINTOR, J., ROTLLAN, P., TORRES, M. & MIRAS-PORTUGAL, M.T. (1992b). Characterization and quantification of diadenosine hexaphosphate in chromaffin cells: Granular storage and secretagogue-induced release. *Anal. Biochem.*, **200**, 296–300.

PINTOR, J., TORRES, M. & MIRAS-PORTUGAL, M.T. (1991). Carbachol induced release of diadenosine polyphosphates-Ap_4A and -Ap_5A from perfused bovine adrenal medulla and isolated chromaffin cells. *Life Sci.*, **48**, 2317–2324.

RODRIGUEZ DEL CASTILLO, A., TORRES, M., DELICADO, E.G. & MIRAS-PORTUGAL, M.T. (1988). Subcellular distribution studies of diadenosine polyphosphates -Ap_4A and -Ap_5A in bovine adrenal medulla: presence in chromaffin granules. *J. Neurochem.*, **51**, 1696–1703.

USUNE, S., KATSURAGI, T. & FURUKAWA, T. (1989). Two phase of the prostaglandin $F_{2\alpha}$-induced contraction in guinea-pig taenia coli involve different Ca^{2+} channels. *Naunyn-Schmied. Arch. Pharmacol.*, **340**, 437–441.

USUNE, S., KATSURAGI, T., SAKAMOTO, Y. & FURUKAWA, T. (1986). Contribution of Na^+ and membrane depolarization to contraction induced by adrenaline in the guinea-pig vas deferens. *Can. J. Physiol. Pharmacol.*, **64**, 720–723.

VON KÜGELGEN, I., BÜLTMANN, R. & STARKE, K. (1990). Interaction of adenine nucleotides, UTP and suramin in mouse vas deferens: suramin-sensitive and suramin-insensitive components in the contractile effect of ATP. *Naunyn-Schmied. Arch. Pharmacol.*, **342**, 198–205.

ZIGANSHIN, A.U., HOYLE, C.H.V., BO, X., LAMBRECHT, G., MUTSCHLER, E., BÄUMERT, H.G. & BURNSTOCK, G. (1993). PPADS selectively antagonizes P_{2X}-purinoceptor-mediated responses in the rabbit urinary bladder. *Br. J. Pharmacol.*, **110**, 1491–1495.

ZIGANSHIN, A.U., HOYLE, C.H.V., LAMBRECHT, G., MUTSCHLER, E., BÄUMERT, H.G. & BURNSTOCK, G. (1994). Selective antagonism by PPADS at P_{2X}-purinoceptors in rabbit isolated blood vessels. *Br. J. Pharmacol.*, **111**, 923–929.

(Received July 17, 1995
Revised October 2, 1995
Accepted October 23, 1995)