ELSEVIER

European Journal of Pharmacology 422 (2001) 197–202

ejp

www.elsevier.nl/locate/ejphar

Nucleotide-evoked relaxation of rat vas deferens—a possible role for endogenous ATP released upon α_1-adrenoceptor stimulation

Ralph Bültmann *, Klaus Starke

Pharmakologisches Institut, Hermann-Herder-Strasse 5, D-79104 Freiburg i.Br., Germany

Received 28 December 2000; received in revised form 16 May 2001; accepted 22 May 2001

Abstract

The possibility was tested that endogenous ATP released upon α_1-adrenoceptor activation causes relaxation of the rat vas deferens smooth muscle. ATP, 2-methylthio ATP and adenosine relaxed the vas deferens precontracted with 80 mM K^+. The metabolically stable P2 receptor agonists α,β-methylene ATP (α,β-MeATP) and adenosine 5′-*O*-(2-thiodiphosphate) (ADPβS) had little or no effect. The adenosine P1 receptor antagonist 8-(*para*-sulfophenyl)theophylline did not significantly affect the response to ATP. The P2 receptor antagonist reactive blue 2 markedly reduced the relaxation (by up to 73%); suramin, pyridoxalphosphate-6-azophenyl-2′,4′-disulphonic acid (PPADS) and acid blue 129 caused no change. ATP, but not α,β-MeATP, also attenuated contractions elicited by noradrenaline at resting tension; reactive blue 2 blocked the inhibitory effect of ATP. Reactive blue 2, by itself, enhanced the response to noradrenaline (by up to 36%); suramin, PPADS and acid blue 129 caused no change. In the presence of the ATP-degrading enzymes apyrase and nucleotide pyrophosphatase, the facilitatory effect of reactive blue 2 was lost. Apyrase, by itself, enhanced the response to noradrenaline (by 13%). The results indicate that endogenous ATP, released from rat vas deferens smooth muscle upon α_1-adrenoceptor stimulation, causes relaxation. The site of action of ATP is not a typical smooth muscle P2Y receptor.

Keywords: Vas deferens, rat; Smooth muscle relaxation; Co-transmission; ATP; P2 receptor; Noradrenaline; α_1-Adrenoceptor

1. Introduction

ATP is a co-transmitter with noradrenaline in postganglionic sympathetic neurones (for review, see Burnstock, 1990; Von Kügelgen and Starke, 1991). In accord with this concept, electrical field stimulation of sympathetically innervated tissue causes an overflow of ATP (e.g. Kurz et al., 1994). Even the first investigators realized that this overflow is not purely neural in origin: a considerable fraction of ATP originates from postjunctional, e.g. smooth muscle, sites (Westfall et al., 1978; Fredholm et al., 1982; Levitt and Westfall, 1982). Subsequent studies clearly demonstrated a release of ATP from smooth muscle tissues upon stimulation of α_1-adrenergic and muscarinic receptors (Westfall et al., 1987; Vizi and Burnstock, 1988; Katsuragi et al., 1990a; Vizi et al., 1992; Kurz et al., 1994). Although the phenomenon is now well documented, the physiological effect, if any, of this non-neural release of ATP is still unknown.

Based on the observation that nifedipine, a known L-type Ca^{2+} channel blocker, but also a putative nucleotide P2 receptor antagonist (Katsuragi et al., 1990b), reduced contractions of the guinea-pig vas deferens elicited by exogenous noradrenaline and the α_1-adrenoceptor agonist methoxamine, Vizi et al. (1992) suggested that ATP released upon α_1-adrenoceptor activation causes contraction of the tissue (see also Katsuragi et al., 1990a). This conclusion seems questionable, since the inhibition by nifedipine might entirely be due to the blockade of L-type Ca^{2+} channels directly activated by α_1-adrenoceptor stimulation (Nelson et al., 1988; Khoyi et al., 1993). Moreover, in the vas deferens of the rat, the P2 receptor antagonists suramin, 4,4′-diisothiocyanatostilbene-2,2′-disulphonate (DIDS) and trypan blue did not change the contraction caused by exogenous noradrenaline, while successfully abolishing the contraction elicited by neurally released ATP (i.e. the purinergic component of the neurogenic contraction; Mallard et al., 1992; Bültmann and Starke, 1994; Bültmann et al., 1994; Kurz et al., 1994).

ATP and related nucleotides are well known to cause contraction of the isolated vas deferens (e.g. Fedan et al., 1982; Bültmann et al., 1999). More recently, *relaxation* of

* Corresponding author. Tel.: +49-761-203-5313; fax: +49-761-203-5318.

E-mail address: bueltman@pironet.de (R. Bültmann).

0014-2999/01/$ - see front matter
PII: S0014-2999(01)01086-X

the vas deferens by ATP has been demonstrated after the tone of the preparation had been raised by high potassium (K^+ 140 mM; Boland et al., 1992; Gailly et al., 1993). Consequently, the present experiments were designed to test the possibility that ATP released upon α_1-adrenoceptor stimulation (Fredholm et al., 1982; Vizi and Burnstock, 1988; Kurz et al., 1994) causes smooth muscle relaxation —the opposite of the effect suggested by Vizi et al. (1992).

2. Materials and methods

2.1. General

Male Wistar rats (220–360 g) were killed by decapitation and the vasa deferentia removed, cleaned of adherent tissue and suspended vertically in a 6.1-ml organ bath. The lower end was fixed and the upper end attached to an isometric force transducer (K30, Hugo Sachs Elektronik, Hugstetten) under an initial tension of 9.8 mN (Graphtec thermal pen recorder, Ettlingen). Unless stated otherwise, the bath fluid was replaced every 15 min. The medium contained (mM): NaCl 118, KCl 4.8, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 0.9, $NaHCO_3$ 25, glucose 11, ascorbic acid 0.3 and disodium EDTA 0.03. It was saturated with 95% O_2/5% CO_2 and kept at 37 °C. Tissues relaxed to about 3 mN during a 60 min equilibration period. This final resting tension remained constant for the remainder of the experiments.

In order to induce a tonic contraction of the vas deferens, the concentration of K^+ in the medium was increased to 80 mM by isomolar replacement of NaCl with KCl ('precontraction'; cf. Boland et al., 1992). The first two additions of K^+, 60 and 120 min after beginning of the experiment, did not yield a stable plateau. Only the following precontractions, obtained at 30-min intervals, served to determine agonist-induced relaxation. Agonists (adenosine or nucleotides) were administered at a single concentration during the plateau of the K^+ response, i.e. about 10 min after the addition of K^+. Only one agonist was tested on each preparation. Agonists were washed out together with K^+ when the relaxation was maximal. Relaxations were measured at their maximum and expressed as a percentage of the respective K^+ contraction.

Contractions elicited by noradrenaline were studied at resting tension. Noradrenaline was added to the medium at 30-min intervals and washed out after the ensuing contraction had peaked. Maximum contraction amplitudes were evaluated. Further experimental details are given in Section 3.

2.2. Statistics

Data are expressed as the arithmetic mean ± S.E.M. Means were tested for a significant difference by the Mann–Whitney test, with Bonferroni correction if applicable. $P < 0.05$ was taken as the limit of statistical significance.

2.3. Materials

Suramin (Bayer, Wuppertal, Germany); 2-methylthio ATP tetrasodium (MeSATP), 8-(*para*-sulfophenyl)theophylline (8-SPT), reactive blue 2 (Biotrend, Köln, Germany); pyridoxalphosphate-6-azophenyl-2′,4′-disulphonic acid (PPADS; Cookson, Southampton, UK); (−)-propranolol hydrochloride (Merck; Darmstadt, Germany); adenosine, adenosine 5′-*O*-(2-thiodiphosphate) trilithium (ADPβS), adenosine 5′-triphosphate disodium (ATP), apyrase (adenosine 5′-triphosphatase and adenosine 5′-diphosphatase; EC 3.6.1.5; grade III), α,β-methylene ATP dilithium (α,β-MeATP), (−)-noradrenaline bi-(+)-tartrate and nucleotide pyrophosphatase (dinucleotide nucleotidohydrolase; EC 3.6.1.9; type III) (Sigma, Deisenhofen, Germany) were dissolved in distilled water or medium. Acid blue 129 (Aldrich, Steinheim, Germany; purified by column chromatography; Tuluc et al., 1998) was dissolved in dimethylsulphoxide (DMSO; final concentration below 0.1%). Solutions of drugs were added to the organ bath in aliquots not exceeding 100 μl.

3. Results

3.1. Relaxation caused by nucleotides and adenosine

Increasing the concentration of K^+ in the bath fluid from 5.7 to 80 mM caused a biphasic contraction of the rat vas deferens (Fig. 1; cf. Triggle et al., 1979; Hay and Wadsworth, 1982); an initial transient phase was followed by a secondary tonic contraction (12.8 ± 0.6 mN; $n = 50$). The latter remained stable while the concentration of K^+ remained elevated (except during the first two—discarded —exposures to 80 mM K^+; see Section 2).

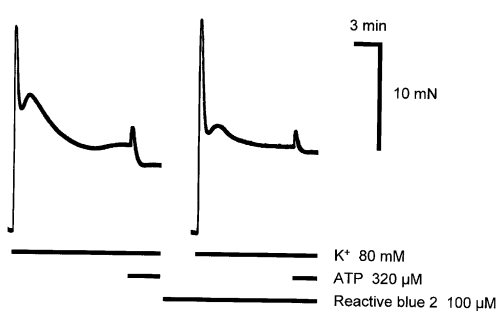

Fig. 1. Relaxation caused by ATP and effect of reactive blue 2: original recordings. K^+ (80 mM) was added to the medium at 30-min intervals and ATP (320 μM) was administered during the plateau of each response to K^+. Reactive blue 2 was added at increasing concentrations (1–100 μM; 0.5-log unit increments) after the second and all following responses to ATP. Shown are responses before addition of reactive blue 2 (left hand tracing) and in the presence of reactive blue 2 (100 μM; right hand tracing). Representative tracings from five experiments.

ATP (320 μM), when added during the plateau of the response to K^+, caused a fast and transient contraction, followed by a slower and sustained relaxation ($19 \pm 1\%$; $n = 29$; all first additions pooled; Fig. 1; cf. Boland et al., 1992). 2-Methylthio ATP (320 μM), likewise, caused contraction followed by relaxation ($23 \pm 2\%$; $n = 4$). The metabolically stable P2 receptor agonists α,β-methylene ATP (α,β-MeATP; 320 μM) and adenosine 5′-*O*-(2-thiodiphosphate) (ADPβS; 320 μM), on the other hand, caused contraction but little (α,β-MeATP; $4 \pm 1\%$) or no (ADPβS) relaxation ($n = 4$ each). Adenosine (320 μM) elicited no contraction, but only rapid and sustained relaxation of the K^+-precontracted vas deferens ($23 \pm 2\%$; $n = 13$; all first additions pooled).

Upon repeated addition of ATP (320 μM) at 30-min intervals, the relaxation increased slightly from the first to the second exposure but remained constant thereafter ($n = 7$). The P2 receptor antagonist reactive blue 2, when added at increasing concentrations after the second addition of ATP, progressively reduced the relaxation caused by ATP (Figs. 1 and 2; maximal inhibition by $73 \pm 2\%$). In contrast, suramin, pyridoxalphosphate-6-azophenyl-2′,4′-disulphonic acid (PPADS) and acid blue 129 caused no change at up to 100 μM (Fig. 2).

Suramin (100 μM) and PPADS (100 μM) completely abolished the transient contractile response preceding the relaxation caused by ATP; reactive blue 2 (100 μM) reduced it by about 50%; acid blue 129 (100 μM) caused no change.

The adenosine P1 receptor antagonist 8-(*para*-sulfophenyl)theophylline (8-SPT; 100 μM) slightly, but not significantly, reduced the relaxation caused by ATP (by $14 \pm 6\%$; $n = 4$; $P > 0.05$); 8-SPT (320 μM) caused no further change ($n = 3$). When tested in a similar protocol, 8-SPT (100 and 320 μM) markedly attenuated the relaxation caused by adenosine (320 μM; by $44 \pm 8\%$ and $79 \pm 6\%$, respectively; $n = 3$ each and 3 controls).

Reactive blue 2 (100 μM) slightly reduced the plateau of the contraction elicited by potassium (by 15%); lower concentrations of reactive blue 2 and all other compounds caused no change.

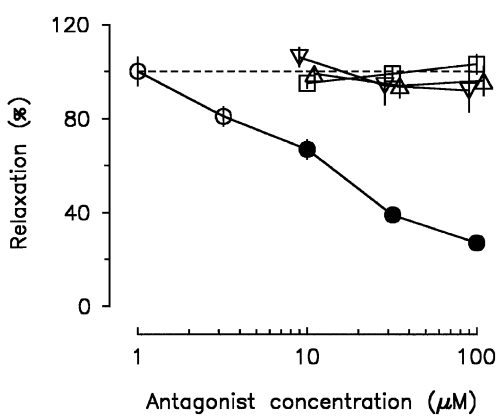

Fig. 2. Effect of P2 receptor antagonists on the relaxation caused by ATP: statistical evaluation. K^+ (80 mM) was added to the medium at 30-min intervals and ATP (320 μM) was administered during the plateau of each response to K^+. Reactive blue 2 (○), suramin (△), PPADS (□) and acid blue 129 (▽) were added at increasing concentrations after the second and all following responses to ATP. Ordinates show relaxation in the presence of antagonists, expressed as a percentage of pre-antagonist values and corrected for average changes observed in solvent controls. Abscissae, antagonist concentration. Filled symbols indicate a significant difference from controls (no antagonist; $P < 0.05$). Means ± S.E.M. from three to five experiments.

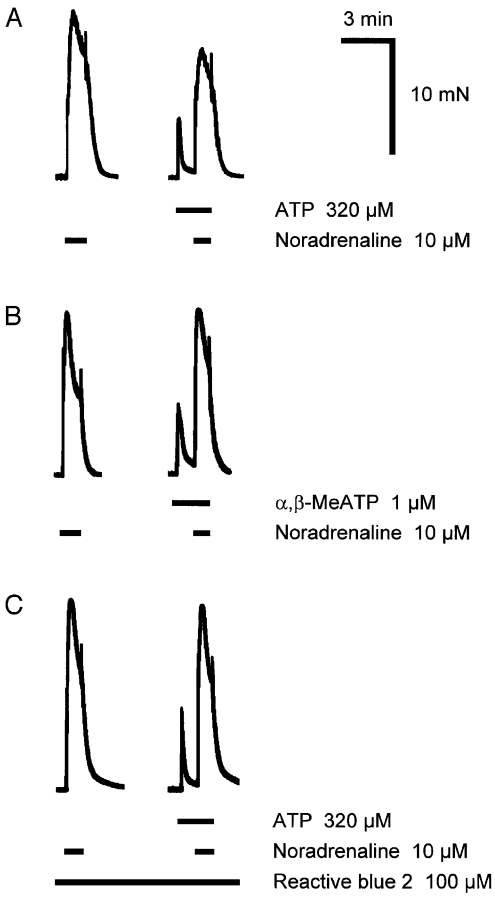

Fig. 3. Effect of ATP, α,β-methylene ATP (α,β-MeATP) and reactive blue 2 on the contraction elicited by noradrenaline: original recordings. Noradrenaline (10 μM) was added to the medium at 30-min intervals at resting tension. ATP (320 μM; A and C) or α,β-MeATP (1 μM; B) was administered 1 min before and during the third addition of noradrenaline. In some experiments (C), the medium contained reactive blue 2 (100 μM) from the beginning. Shown are responses to the second (left hand tracings) and third addition of noradrenaline (right hand tracings). Representative tracings from four to five experiments.

3.2. Contraction elicited by noradrenaline

Noradrenaline (10 μM), added at resting tension, caused contraction of the rat vas deferens amounting to 13.8 ± 0.2 mN ($n = 94$; all first additions in the absence of further drugs pooled; Fig. 3). Upon repeated addition at 30-min intervals, the contraction increased slightly from the first to

the second exposure but remained constant thereafter ($n = 8$).

ATP (320 μM), added 1 min before the third addition of noradrenaline, elicited a transient contraction of the vas deferens (5.6 ± 0.5 mN; $n = 5$). In the continued presence of ATP the subsequent response to noradrenaline was reduced (by $18 \pm 2\%$; $P < 0.05$; Fig. 3A). α,β-MeATP (1 μM) caused a contraction of similar height (8.1 ± 0.7 mN; $n = 4$), but did not alter the response to noradrenaline (Fig. 3B). Adenosine (320 μM) did not, by itself, change the resting tension of the vas deferens, but reduced the subsequent response to noradrenaline (by $14 \pm 1\%$; $n = 3$; $P < 0.05$).

When the medium contained reactive blue 2 (100 μM) from the beginning, the first contraction elicited by noradrenaline was increased (19.5 ± 0.7 mN; $n = 5$; $P < 0.05$). The contraction elicited by ATP, in contrast, remained unchanged (6.8 ± 0.6 mN; cf. Bültmann and Starke, 1994; Bültmann et al., 1999). In the presence of reactive blue 2 (100 μM), prior addition of ATP did not alter the contraction elicited by noradrenaline (Fig. 3C).

When the medium contained 8-SPT (320 μM) from the beginning, the first contraction elicited by noradrenaline remained unchanged (14.9 ± 0.5 mN; $n = 6$), as did the contraction elicited by ATP (6.8 ± 1.3 mN; $n = 3$). In the presence of 8-SPT (320 μM), prior addition of ATP still reduced the contraction elicited by noradrenaline (by $24 \pm 3\%$; $P < 0.05$); adenosine (320 μM), in contrast, had little effect (reduction by $3 \pm 1\%$; $n = 3$; $P < 0.05$ versus adenosine alone).

The interaction of reactive blue 2 with noradrenaline was analyzed in greater detail. The results are summarized in Fig. 4 and Table 1.

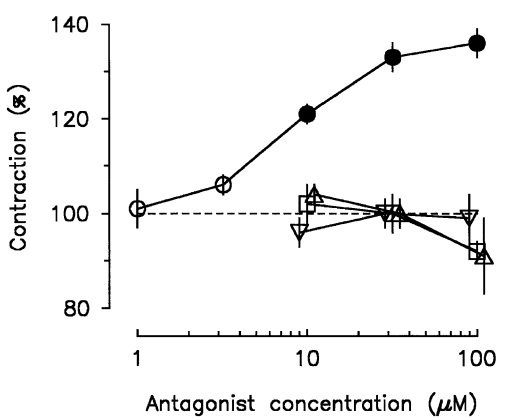

Fig. 4. Effect of P2 receptor antagonists on the contraction elicited by noradrenaline: statistical evaluation. Noradrenaline (10 μM) was added to the medium at 30-min intervals at resting tension. Reactive blue 2 (○), suramin (△), PPADS (□) and acid blue 129 (▽) were added at increasing concentrations after the second and all following responses to noradrenaline. Ordinates show contraction in the presence of antagonists, expressed as a percentage of pre-antagonist values and corrected for average changes observed in solvent controls. Abscissae, antagonist concentration. Filled symbols indicate a significant difference from controls (no antagonist; $P < 0.05$). Means ± S.E.M. from three to five experiments.

Table 1
Effect of various compounds, alone or in combination with reactive blue 2, on the contraction elicited by noradrenaline

Compound present during S_3	Contraction elicited by noradrenaline (S_3/S_2)	
	Without addition of reactive blue 2	Reactive blue 2 (10 μM) present during S_3
Solvent	1.00 ± 0.01	1.29 ± 0.04[a,b]
Propranolol (10 μM)	1.23 ± 0.03[a]	1.49 ± 0.05[a,b]
8-SPT (100 μM)	0.99 ± 0.01	1.25 ± 0.05[a,b]
Apyrase (1 U/ml)	1.01 ± 0.02	1.25 ± 0.01[a,b]
Apyrase (10 U/ml)	1.13 ± 0.03[a]	1.18 ± 0.06[a]
Nucleotide pyrophosphatase (1 U/ml)	0.60 ± 0.04[a]	0.60 ± 0.05[a]

Noradrenaline (10 μM) was added to the medium three times, interval of 30 min (S_1–S_3). Test compounds were added, either alone or in combination with reactive blue 2, immediately after the second (S_2) and during the third addition of noradrenaline (S_3). Shown are responses to the third addition of noradrenaline expressed as a percentage of the response to the second addition (S_3/S_2). Means ± S.E.M. from three to seven experiments.

[a] Denotes a significant ($P < 0.05$) difference from control (solvent, without addition of reactive blue 2).

[b] Denotes a significant ($P < 0.05$) difference from the respective group without addition of reactive blue 2.

When added at increasing concentrations after the second addition of noradrenaline, reactive blue 2 progressively enhanced the contraction (Fig. 4; maximal increase by $36 \pm 3\%$). Suramin, PPADS and acid blue 129, in contrast, caused no change at up to 100 μM (Fig. 4).

In order to test the possibility that reactive blue 2 enhanced the contraction elicited by noradrenaline by preventing a β-adrenoceptor or adenosine receptor mediated relaxation, effects of propranolol and 8-SPT were studied. Propranolol (10 μM), when added alone, increased the contraction of the vas deferens caused by noradrenaline (cf. Kidman et al., 1979; Suzuki et al., 1986); 8-SPT (100 μM) caused no change (Table 1). In the presence of propranolol (10 μM) or 8-SPT (100 μM), reactive blue 2 (10 μM) still augmented the response to noradrenaline (Table 1).

Finally, two ATP-degrading enzymes, apyrase and nucleotide pyrophosphatase, were used in order to test the possibility that blockade of a relaxant effect of endogenous ATP (released by noradrenaline upon α_1-adrenoceptor stimulation; see Section 1) contributes to the facilitatory effect of reactive blue 2. At a concentration of 1 unit (U) per ml, apyrase did not alter the contraction caused by noradrenaline; at a higher concentration (10 U/ml), the enzyme caused a significant increase (by 13%; Table 1). Nucleotide pyrophospatase (1 U/ml) alone caused small irregular contractions of the vas deferens and markedly reduced the response to noradrenaline (Table 1). In the presence of apyrase (1 U/ml), reactive blue 2 (10 μM)

still augmented the contraction caused by noradrenaline; however, in the presence of apyrase (10 U/ml) or nucleotide pyrophosphatase (1 U/ml), the facilitation by reactive blue 2 was lost (Table 1).

4. Discussion

Our results confirm the initial observation by Boland et al. (1992): extracellular nucleotides cause relaxation of the vas deferens after the tone of the preparation has been raised by high K^+. The response to ATP was not significantly affected by the P1 receptor antagonist 8-SPT (Bruns et al., 1986), given at a concentration (320 μM) that almost abolished the response to adenosine, indicating that the nucleotide acts per se and not after degradation to adenosine. Based on experiments in the vas deferens of the mouse, Boland et al. (1992) suggested that ATP activates a relaxation-mediating smooth muscle P_{2Y} purinoceptor. In the vas deferens of the rat this seems unlikely because ADPβS, which is known to activate P_{2Y} purinoceptors (i.e. $P2Y_1$ receptors; see Fredholm et al., 1997; Harden et al., 1998 for the nomenclature of P2Y receptors) in other smooth muscle preparations (e.g. guinea-pig taenia coli; Bültmann et al., 1996), failed to cause relaxation. Moreover, suramin, PPADS and acid blue 129, which all block P2Y receptors in other tissues (e.g. Bültmann et al., 1996; Johnson et al., 1996; Tuluc et al., 1998), did not attenuate the relaxation caused by ATP (Fig. 2).

ATP (320 μM) also attenuated the contraction of the rat vas deferens elicited by noradrenaline at resting tension (Fig. 3A). This finding is in contrast to previous studies showing either no effect (Clanachan et al., 1977) or a slight augmentation by ATP (Huidobro-Toro and Parada, 1988). The discrepancy may be due to different protocols and experimental conditions. The inhibitory effect of ATP was not mimicked by α,β-MeATP (at a concentration equieffective at causing contraction of the vas deferens; Fig. 3B) and was lost in the presence of reactive blue 2 (100 μM; Fig. 3C), indicating that it was independent of the ATP-evoked contraction. The underlying mechanism is likely to be the one mediating the relaxation caused by ATP during exposure to high K^+ (see above).

Irrespective of the site of action, some findings of the present study indicate that, like exogenous nucleotides, *endogenous* ATP also causes smooth muscle relaxation.

Noradrenaline releases ATP from non-neural, presumably smooth muscle, sites in rat vas deferens (Fredholm et al., 1982; Vizi and Burnstock, 1988; Kurz et al., 1994). This noradrenaline-induced release of ATP is exclusively due to activation of α_1-adrenoceptors and subsequent influx of Ca^{2+} through L-type Ca^{2+} channels (Kurz et al., 1994; see also Khoyi et al., 1993). In the present study, the contraction of the vas deferens elicited by noradrenaline remained unchanged in the presence of the P2 receptor antagonists suramin, PPADS and acid blue 129 (Fig. 4; see Section 1), which all also did not attenuate the relaxation caused by ATP (Fig. 2). Reactive blue 2, on the other hand, augmented the response to noradrenaline (Figs. 3 and 4), and did so over the same range of concentrations at which it also attenuated the relaxation caused by exogenous ATP (Fig. 2). Taken together, these findings indicate that reactive blue 2 might increase the contraction in response to noradrenaline by preventing a relaxation caused by concomitantly released *endogenous* ATP.

The enhancement by reactive blue 2 was preserved in the presence of the β-adrenoceptor antagonist propranolol (Table 1), indicating that reactive blue 2 did not act by blocking the β-adrenoceptor-mediated relaxation of the tissue (May et al., 1985; Diaz-Toledo and Jurkiewicz, 1991). The P1 receptor antagonist 8-SPT, likewise, caused no change (Table 1); antagonism against endogenous adenosine (released upon α_1-adrenoceptor stimulation; Fredholm et al., 1982), therefore, cannot explain the facilitatory effect of reactive blue 2. On the other hand, the enhancement was partly or completely lost in the presence of two enzymes known to degrade ATP, apyrase and nucleotide pyrophosphatase (Table 1), supporting the view that the effect of reactive blue 2 is due to an interaction with released endogenous ATP.

Neither enzyme affected the relaxation caused by exogenous ATP (320 μM; R. Bültmann; unpublished observation). This is likely due to the fact that the bulk concentration of agonist in the organ bath is hardly altered during the short period it takes for the response to develop (Fig. 1). Nucleotide pyrophosphatase, by itself, markedly decreased rather than increased the contraction caused by noradrenaline (Table 1), possibly due to a direct action on the smooth muscle, which is also evident from the small contractions observed in the presence of the enzyme (see also Manzini et al., 1985). In contrast, apyrase alone (at a concentration of 10 U/ml) augmented the contraction caused by noradrenaline (Table 1), supporting the hypothesis that endogenous ATP in fact causes smooth muscle relaxation.

We have no reasons to assume that ATP, released upon α_1-adrenoceptor stimulation, also activates excitatory P2X receptors. Suramin and PPADS did not alter the relaxant effect of ATP (Fig. 2), but both antagonists block the P2X receptor-mediated contraction of rat vas deferens elicited by exogenous or neurally released ATP (present study; Mallard et al., 1992; Bültmann et al., 1999). If there was an activation of P2X receptors by released endogenous ATP, suramin and PPADS should have reduced the contraction elicited by noradrenaline. The observation that they did not (Fig. 4) argues against a possible activation of P2X receptors (see Section 1). The reason for the apparently selective activation of the relaxant mechanism is not known. It is tempting to assume a co-localization of the sites of action and the sites of release of ATP on the surface of the smooth muscle cell.

Acknowledgements

We thank Bayer for suramin. This study was supported by the Deutsche Forschungsgemeinschaft (Sta 149/1-2).

References

Boland, B., Himpens, B., Vincent, M.F., Gillis, J.M., Casteels, R., 1992. ATP activates P_{2x}-contracting and P_{2y}-relaxing purinoceptors in the smooth muscle of mouse vas deferens. Br. J. Pharmacol. 107, 1152–1158.

Bruns, R.F., Lu, G.H., Pugsley, T.A., 1986. Characterization of the A_2 adenosine receptor labeled by [^{3}H]NECA in rat striatal membranes. Mol. Pharmacol. 29, 331–346.

Bültmann, R., Starke, K., 1994. Blockade by 4,4′-diisothiocyanatostilbene-2,2′-disulphonate (DIDS) of P_{2X}-purinoceptors in rat vas deferens. Br. J. Pharmacol. 112, 690–694.

Bültmann, R., Trendelenburg, M., Starke, K., 1994. Trypan blue blocks P_{2X}-purinoceptors in rat vas deferens. Br. J. Pharmacol. 113, 349–354.

Bültmann, R., Dudeck, O., Starke, K., 1996. Evaluation of P_2-purinoceptor antagonists at two relaxation-mediating P_2-purinoceptors in guinea-pig taenia coli. Naunyn-Schmiedeberg's Arch. Pharmacol. 353, 445–451.

Bültmann, R., Klebroff, W., Starke, K., 1999. A contraction-mediating receptor for UTP, presumably $P2Y_2$, in rat vas deferens. Naunyn-Schmiedeberg's Arch. Pharmacol. 360, 196–201.

Burnstock, G., 1990. Co-transmission. Arch. Int. Pharmacodyn. 304, 7–33.

Clanachan, A.S., Johns, A., Paton, D.M., 1977. Presynaptic inhibitory actions of adenine nucleotides and adenosine on neurotransmission in the rat vas deferens. Neuroscience 2, 597–602.

Diaz-Toledo, A., Jurkiewicz, A., 1991. Different mechanisms of action of agents acting on β-adrenoceptors in barium-stimulated and electrically stimulated rat vas deferens. Br. J. Pharmacol. 104, 277–283.

Fedan, J.S., Hogaboom, G.K., Westfall, D.P., O'Donnell, J.P., 1982. Comparison of contractions of the smooth muscle of the guinea-pig vas deferens induced by ATP and related nucleotides. Eur. J. Pharmacol. 81, 193–204.

Fredholm, B.B., Fried, G., Hedqvist, P., 1982. Origin of adenosine released from rat vas deferens by nerve stimulation. Eur. J. Pharmacol. 79, 233–243.

Fredholm, B.B., Abbracchio, M.P., Burnstock, G., Dubyak, G.R., Harden, T.K., Jacobson, K.A., Schwabe, U., Williams, M., 1997. Towards a revised nomenclature for P1 and P2 receptors. Trends Pharmacol. Sci. 18, 79–82.

Gailly, P., Boland, B., Paques, C., Himpens, B., Casteels, R., Gillis, J.M., 1993. Post-receptor pathway of the ATP-induced relaxation in smooth muscle of the mouse vas deferens. Br. J. Pharmacol. 110, 326–330.

Harden, T.K., Barnard, E.A., Boeynaems, H.M., Burnstock, G., Dubyak, G., Hourani, S.M.O., Insel, P.A., 1998. P2Y receptors. The IUPHAR Compendium of Receptor Characterization and Classification. IUPHAR Media, London, pp. 209–217.

Hay, D.W.P., Wadsworth, R.M., 1982. Effects of some organic calcium antagonists and other procedures affecting Ca^{2+} translocation on KCl-induced contractions in the rat vas deferens. Br. J. Pharmacol. 76, 103–113.

Huidobro-Toro, P.J., Parada, S., 1988. Co-transmission in the rat vas deferens: postjunctional synergism of noradrenaline and adenosine 5′-triphosphate. Neurosci. Lett. 85, 339–344.

Johnson, C.R., Charlton, S.J., Hourani, S.M.O., 1996. Responses of the longitudinal muscle and the muscularis mucosae of the rat duodenum to adenine and uracil nucleotides. Br. J. Pharmacol. 117, 823–830.

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.

Khoyi, M.A., Dalziel, H.H., Zhang, L., Bjur, R.A., Gerthoffer, W.T., Buxton, I.L.O., Westfall, D.P., 1993. $[Ca^{2+}]_i$-sensitive, IP_3-independent Ca^{2+} influx in smooth muscle of rat vas deferens revealed by procaine. Br. J. Pharmacol. 110, 1353–1358.

Kidman, C.D., Mottram, D.R., Hickman, J.A., 1979. Potentiation of the response of rat vas deferens to noradrenaline by dicyclohexylamine and related amines. Arch. Int. Pharmacodyn. 238, 180–186.

Kurz, A.K., Bültmann, R., Driessen, B., Von Kügelgen, I., Starke, K., 1994. Release of ATP in rat vas deferens: origin and role of calcium. Naunyn-Schmiedeberg's Arch. Pharmacol. 350, 491–498.

Levitt, B., Westfall, D.P., 1982. Factors influencing the release of purines and norepinephrine in the rabbit portal vein. Blood Vessels 19, 30–40.

Mallard, N., Marshall, R., Sithers, A., Spriggs, B., 1992. Suramin: a selective inhibitor of purinergic neurotransmission in rat isolated vas deferens. Eur. J. Pharmacol. 220, 1–10.

Manzini, S., Maggi, C.A., Meli, A., 1985. Further evidence for involvement of adenosine−triphosphate in non-adrenergic non-cholinergic relaxation of the isolated rat duodenum. Eur. J. Pharmacol. 113, 399–408.

May, J.M., Abel, P.W., Minneman, K.P., 1985. Binding of agonists and antagonists to β-adrenoceptors in rat vas deferens: relationship to functional response. Naunyn-Schmiedeberg's Arch. Pharmacol. 331, 324–333.

Nelson, M.T., Standen, M.B., Brayden, J.E., Worley, J.F., 1988. Noradrenaline contracts arteries by activating voltage-dependent calcium channels. Nature 336, 382–385.

Suzuki, N., Inagaki, O., Kasuya, Y., 1986. Inhibitory action of propranolol on the contractions induced by nerve stimulations or calcium in the smooth muscle of rat vas deferens. Jpn. J. Pharmacol. 40, 455–462.

Triggle, C.R., Swamy, V.C., Triggle, D.J., 1979. Calcium antagonists and contractile responses in rat vas deferens and guinea pig ileal smooth muscle. Can. J. Physiol. Pharmacol. 57, 804–818.

Tuluc, F., Bültmann, R., Glänzel, M., Frahm, A.W., Starke, K., 1998. P2-receptor antagonists: IV. Blockade of P2-receptor subtypes and ecto-nucleotidases by compounds related to reactive blue 2. Naunyn-Schmiedeberg's Arch. Pharmacol. 357, 111–120.

Vizi, E.S., Burnstock, G., 1988. Origin of ATP release in the rat vas deferens: concomitant measurement of [^{3}H]noradrenaline and [^{14}C]ATP. Eur. J. Pharmacol. 158, 69–77.

Vizi, E.S., Sperlágh, B., Baranti, M., 1992. Evidence that ATP, released from the postsynaptic site by noradrenaline, is involved in mechanical responses of guinea-pig vas deferens: cascade transmission. Neuroscience 50, 455–465.

Von Kügelgen, I., Starke, K., 1991. Noradrenaline-ATP co-transmission in the sympathetic nervous system. Trends Pharmacol. Sci. 12, 319–324.

Westfall, D.P., Stitzel, R.E., Rowe, J.N., 1978. The postjunctional effects and neural release of purine compounds in the guinea-pig vas deferens. Eur. J. Pharmacol. 50, 27–38.

Westfall, D.P., Sedaa, K., Bjur, R.A., 1987. Release of endogenous ATP from rat caudal artery. Blood Vessels 24, 125–127.