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On the suitability of adenosine 3′-phosphate 5′-phosphosulphate as a selective P2Y receptor antagonist in intact tissues

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Abstract

Agonist and antagonist effects of the putative $P2Y_1$ receptor antagonist adenosine 3′-phosphate 5′-phosphosulphate (PAPS) were studied in intact tissues. In the carbachol-precontracted guinea-pig taenia coli, PAPS caused prominent relaxation (EC_{50} 3.3 μM). The response was attenuated by the P2 receptor antagonists 4,4′-diisothiocyanatostilbene-2,2′-disulphonate (DIDS) and reactive red 2 with apparent K_d values (0.27 and 0.29 μM) indicating that PAPS acts through the non-P2Y receptor, which is the site of action of α,β-methylene ATP (α,β-MeATP) in the taenia coli. Incubation with PAPS (10–100 μM) attenuated the P2Y receptor-mediated relaxation caused by 5′-*O*-(2-thiodiphosphate) (ADPβS); PAPS (100 μM) also attenuated the relaxation caused by α,β-MeATP, as well as the α_1-adrenoceptor-mediated response to noradrenaline. In the noradrenaline-precontracted rat aorta, PAPS caused minor relaxation (EC_{50} 24.7 μM), which was reduced by the P2 receptor antagonist pyridoxal-phosphate-6-azophenyl-2′,5′-disulphonate (*iso*-PPADS; 1 μM), indicating that PAPS activates endothelial P2Y receptors. Incubation with PAPS (10 and 100 μM) attenuated the P2Y receptor-mediated relaxation caused by ADPβS; PAPS (100 μM) also attenuated the P2U receptor-mediated relaxation caused by UTP and the muscarine receptor-mediated response to acetylcholine. In rat vas deferens, PAPS (100 μM) attenuated the P2X receptor-mediated contraction elicited by α,β-MeATP but did not alter the α_1-adrenoceptor-mediated response to noradrenaline. The results indicate that PAPS attenuates P2Y receptor-mediated relaxation in intact tissues. However, due to its limited subtype selectivity and non-P2 receptor effects, the nucleotide is not a suitable antagonist for this subtype.

Keywords: Taenia coli, guinea-pig; Aorta, rat; Vas deferens, rat; P2 receptor antagonist; P2X receptor; P2Y receptor; P2U receptor; Adenosine 3′-phosphate 5′-phosphosulphate

1. Introduction

During the past few years, the coding sequences of several membrane receptors for extracellular nucleotides have been identified. On the basis of their different molecular structure, two receptor families are currently discerned: P2X receptors ($P2X_{1-7}$), which form ligand-gated ion channels, and G-protein-coupled P2Y receptors ($P2Y_{1-7}$; see Fredholm et al., 1997 for review). Upon heterologous expression, the cloned P2Y receptor subtypes can be discriminated by means of agonist potency series, provided care is taken to avoid degradation or interconversion of the nucleotides employed (see Harden et al., 1997). The recombinant $P2Y_1$ receptor, for example, is particularly sensitive to 2-methylthio ATP (MeSATP) but insensitive to UTP; it is thought to represent the ‘classical’ P2Y receptors [1] previously identified pharmacologically in the guinea-pig taenia coli and in vascular endothelium (see Burnstock and Kennedy, 1985; O’Connor et al., 1991; Fredholm et al., 1994).

P2 receptor research is impeded by the lack of specific and subtype-selective antagonists. For this reason, the recent introduction of adenosine 3′-phosphate 5′-phosphosulphate (PAPS; known as a sulphate donor in biotransformation reactions) and adenosine 3′-phosphate 5′-phosphate as selective $P2Y_1$ receptor antagonists might be an

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[1] The terms like P2Y and P2X are used here to designate native receptors that have been defined pharmacologically (see Fredholm et al., 1994), whereas $P2X_n$ and $P2Y_n$ refer to the molecularly defined receptor subtypes (see Fredholm et al., 1997).

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important step ahead. Both compounds behaved as weak partial agonists at the P2Y receptor of turkey erythrocytes ($P2Y_1$; apparent antagonist K_d values 0.35 and 2.2 μM, respectively), were competitive antagonists devoid of agonist activity at the recombinant human $P2Y_1$ receptor expressed in 1321N1 human astrocytoma cells, and displayed no agonist or antagonist activity at recombinant $P2Y_2$, $P2Y_4$ and $P2Y_6$ subtypes equally expressed in these cells. They did not block the turkey erythrocyte β-adrenoceptor which, like the $P2Y_1$ receptor, couples to the phospholipase C cascade (Boyer et al., 1996).

The present experiments were performed in order to test the suitability of PAPS as a selective P2Y receptor antagonist in intact tissues. Possible agonist and antagonist effects were assessed in three model tissues previously characterized in our laboratory: the guinea-pig taenia coli, the rat aorta and the rat vas deferens. In the carbachol-precontracted guinea-pig taenia coli, nucleotides cause relaxation either by activation of a P2Y receptor, which is the site of action of 5′-*O*-(2-thiodiphosphate) (ADPβS), or through a receptor distinct from P2Y and selectively activated by α,β-methylene ATP (α,β-MeATP; Dudeck et al., 1995; Windscheif et al., 1995; Bültmann et al., 1996; Lambrecht et al., 1996). In the noradrenaline-precontracted rat aorta, the endothelium-dependent relaxation caused by ADPβS is again mediated by a P2Y receptor; UTP selectively acts on a P2U receptor to elicit relaxation (Dainty et al., 1991; Hansmann et al., 1997). In rat vas deferens, α,β-MeATP causes contraction by activation of a P2X receptor (Bültmann and Starke, 1994; Khakh et al., 1994, 1995); the existence of two additional contraction-mediating receptors for ATP, distinct from P2X, has been proposed (Bültmann and Starke, 1994).

Of these native P2 receptors, only the P2X receptor of rat vas deferens has been cloned ($P2X_1$; Valera et al., 1994). The P2Y receptors of the guinea-pig taenia coli and rat aortic endothelium—both 'classical' P2Y (see above) —resemble the native turkey erythrocyte and the recombinant human $P2Y_1$ receptor in terms of agonist potency orders (cf. Schachter et al., 1996), but differ from each other in terms of antagonist sensitivity (see Hansmann et al., 1997). It would have been of interest to study the effects of PAPS also in a tissue with molecularly identified functional $P2Y_1$ receptors. However, no such tissue is known; although a $P2Y_1$ receptor has been cloned from rat ileal myocytes, a functional role of this receptor remains to be defined (Pacaud et al., 1996).

2. Materials and methods

2.1. General

Methods were those of Bültmann and Starke (1995), Dudeck et al. (1995) and Hansmann et al. (1997). Briefly, relaxations of strips of the guinea-pig taenia coli, precontracted with carbachol (50–90 nM), were elicited by PAPS, ADPβS, α,β-MeATP or noradrenaline. Relaxations of rings of the rat aorta, precontracted with noradrenaline (1 μM), were elicited by PAPS, ADPβS, UTP or acetylcholine. Contractions of prostatic portions of the rat vas deferens were elicited by PAPS, α,β-MeATP or noradrenaline. Agents causing relaxation (taenia coli and aorta) or contraction (vas deferens) were washed out after responses had peaked. Two concentration–relaxation curves for PAPS, ADPβS or α,β-MeATP (taenia coli), three concentration–relaxation curves for ADPβS or UTP (aorta) or two concentration–contraction curves for α,β-MeATP (vas deferens) were determined in each experiment. Antagonists or solvent were added after the first curve.

Logistic curves were fitted to the weighted mean relaxation or contraction values by means of equation 25 of Waud (1976) and nonlinear regression. The calculation yielded the maximal agonist effect and the EC_{50}, i.e., the concentration producing 50% of the maximum of that curve. The shift of the second or third curve with respect to the first curve in the presence of an antagonist ('agonist concentration ratio') was read at the level of the EC_{50} and corrected for the mean shift occurring in solvent controls. Apparent antagonist K_d values in guinea-pig taenia coli were calculated using equation 4 of Furchgott (1972).

2.2. Statistics

Data are expressed as either the arithmetic mean $\pm$ S.E.M or, in the case of EC_{50}, values and maximal effects, the S.E. as defined by Waud (1976). Fitted curves were tested for a significant difference according to p. 371 of Motulsky and Ransnas (1987). $P < 0.05$ was taken as the limit of statistical significance.

2.3. Materials

Reactive red 2 was synthesized in our laboratory as previously described; the identity and purity of the product was confirmed by thin layer chromatography, ^{1}H-nuclear magnetic resonance and infrared spectroscopy (Bültmann and Starke, 1995). Other drugs used were pyridoxalphosphate-6-azophenyl-2′,5′-disulphonate tetrasodium (*iso*-PPADS; Cookson, Southampton, UK); acetylcholine chloride, adenosine 3′-phosphate 5′-phosphosulphate, adenosine 5′-*O*-(2-thiodiphosphate) trilithium (ADPβS), carbachol chloride, 4,4′-diisothiocyanatostilbene-2,2′-disulphonate disodium (DIDS), α,β-methylene ATP dilithium (α,β-MeATP), (−)-noradrenaline hydrogen-(+)-tartrate, UTP trisodium (Sigma). Compounds were dissolved in distilled water or medium.

3. Results

3.1. Relaxation of guinea-pig taenia coli

When added during the plateau of the contraction elicited by carbachol (50–90 nM; force of contraction 74 mN on average), PAPS elicited rapid, transient relaxation of the guinea-pig taenia coli (EC_{50} 3.3 ± 0.1 μM; maximal relaxation $101 \pm 1\%$; $n = 14$; empty circles in Fig. 1). In order to characterize the relaxation-mediating receptor, interactions of 4,4′-diisothiocyanatostilbene-2,2′-disulphonate (DIDS; Dudeck et al., 1995) and reactive red 2 (Bültmann and Starke, 1995) with PAPS were assessed. A second concentration–relaxation curve of PAPS, after addition of solvent (water), was close to the first (Fig. 1a). DIDS (10 μM) and reactive red 2 (1 μM) shifted the curve to the right without changing the maximum (Fig. 1b and c), yielding apparent antagonist K_d values of 0.27 and 0.29 μM, respectively. DIDS (100 μM) abolished the relaxation caused by PAPS over the entire range of concentrations studied ($n = 2$; not shown).

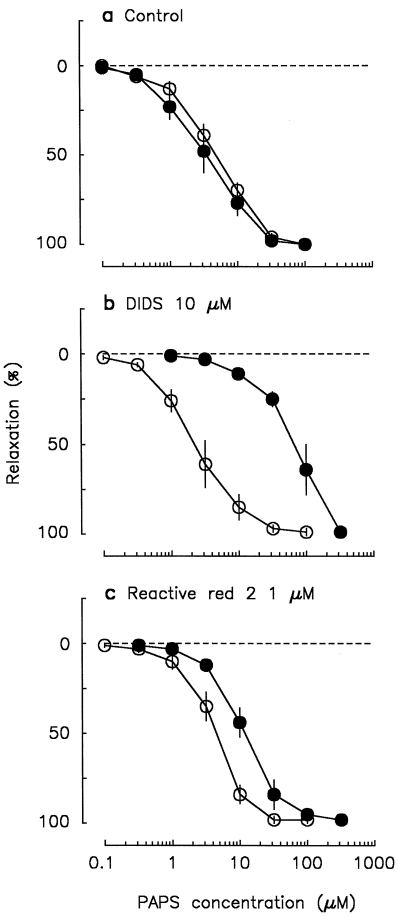

Fig. 1. Guinea-pig taenia coli: relaxation by PAPS and interaction with DIDS and reactive red 2. Carbachol was added every 15 min. Increasing concentrations of PAPS were added with each successive carbachol dose. Two concentration–response curves were determined in each tissue. Solvent (a), DIDS (10 μM; (b)) or reactive red 2 (1 μM; (c)) was added after completion of the first curve (○) and the second curve (●) was determined 60 min later. Abscissae show concentration of PAPS; ordinates show relaxation as a percentage of the respective response to carbachol. Means ± S.E.M. from four experiments.

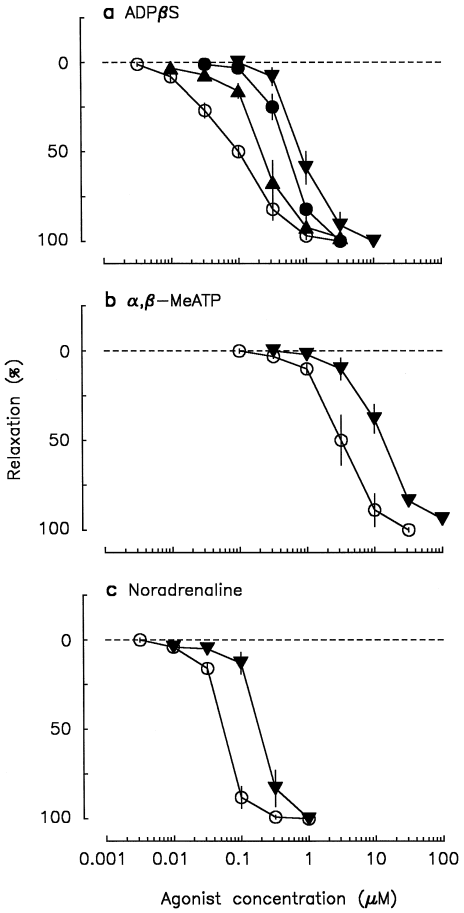

Fig. 2. Guinea-pig taenia coli: effect of PAPS on nucleotide- and noradrenaline-evoked relaxations. Carbachol was added every 15 min. Increasing concentrations of ADPβS (a), α,β-MeATP (b) or noradrenaline (c) were added with each successive carbachol dose. Two concentration–response curves were determined in each tissue. Solvent (○) or PAPS (10 μM ▲; 32 μM ●; 100 μM ▼) was added after completion of the first curve and the second curve was determined 60 min later. Abscissae show agonist concentration; ordinates show relaxation in second concentration–response curves as a percentage of the respective response to carbachol. Means ± S.E.M. from three to eight experiments.

In order to test a possible antagonist effect of PAPS, two concentration–relaxation curves of ADPβS (acting through P2Y receptors), α,β-MeATP (acting through the separate α,β-MeATP-receptors) or noradrenaline (acting through α_1-adrenoceptors; Den Hertog et al., 1984) were determined in each taenia coli strip (cf. Bültmann and Starke, 1995). In the first curve, the EC_{50} (and maximal percentage relaxation) values were 103 ± 17 nM ($101 \pm 3\%$) for ADPβS ($n = 20$), 2.2 ± 0.3 μM ($104 \pm 4\%$) for α,β-MeATP ($n = 7$) and 45 ± 1 nM ($100 \pm 1\%$) for noradrenaline ($n = 7$). For either agonist, a second curve, after addition of solvent (water), was close to the first ($n = 3$ to 8; not shown).

Table 1
Potency of adenosine 3′-phosphate 5′-phosphosulphate (PAPS) at antagonizing effects of various agonists

Tissue	Agonist	Response	Receptor	Agonist concentration ratio in experiments with PAPS		
				10 μM	32 μM	100 μM
Guinea-pig taenia coli	ADPβS	Relaxation	P2Y receptor	3.2	4.8	9.6
	α,β-MeATP	Relaxation	α,β-MeATP-receptor			4.8
	Noradrenaline	Relaxation	α_1-adrenoceptor			3.7
Rat aorta	ADPβS	Relaxation	P2Y receptor	3.5[a]		3.9[a]
	UTP	Relaxation	P2U receptor	0.9		4.2
	Acetylcholine	Relaxation	Muscarine receptor	1.6		2.7[a]
Rat vas deferens	α,β-MeATP	Contraction	P2X receptor	1.3		3.8[a]
	Noradrenaline	Contraction	α_1-Adrenoceptor			1.2

The potency of PAPS is expressed in terms of the agonist concentration ratio, i.e., the ratio of the agonist EC_{50} in the presence of the concentration of PAPS indicated over the EC_{50} in the absence of PAPS. From the experiments of Figs. 2, 4 and 5 and from the noradrenaline experiments in rat vas deferens mentioned in the text.
[a]Maximum of concentration–response curve reduced in presence of PAPS.

PAPS (10, 32 or 100 μM), when added after completion of the first concentration–response curve of ADPβS, α,β-MeATP or noradrenaline, did not change the tension of the (non-precontracted) taenia coli and also did not change subsequent contractions elicited by carbachol. PAPS (10, 32 and 100 μM) shifted the concentration–relaxation curve of ADPβS progressively to the right without changing the maximum (Fig. 2a). The degree of the shift—i.e., the ADPβS concentration ratio—increased with the concentration of PAPS (Table 1). The increase was less, however, than expected from the relationship between antagonist concentration and agonist concentration ratio for the case of competitive antagonism (equation 3 of Furchgott, 1972). In consequence, the apparent antagonist K_d values calculated from the shift increased with the concentration of PAPS (4.6, 8.4 and 11.6 μM, respectively), indicating deviation from competitive kinetics, as in the case of many P2-antagonists (see Bültmann et al., 1996). PAPS (100 μM) also shifted the concentration–relaxation curve of α,β-MeATP and noradrenaline to the right (Fig. 2b and c). As shown by the ensuing concentration ratios, its potency against α,β-MeATP and noradrenaline was hardly less than against ADPβS (Table 1).

3.2. Relaxation of rat aorta

When added during the plateau of the contraction elicited by noradrenaline (1 μM; force of contraction 5.0 mN on average), PAPS elicited weak relaxation of the rat aorta (EC_{50} 24.7 ± 4.0 μM; maximal relaxation 36 ± 2%; $n = 7$; empty circles in Fig. 3). In order to characterize the receptor involved, the interaction of pyridoxalphosphate-6-azophenyl-2′,5′-disulphonate (*iso*-PPADS; Hansmann et al., 1997) with PAPS was examined. A second concentration-relaxation curve of PAPS, after addition of solvent (water), was close to the first (Fig. 3a). *iso*-PPADS (1 μM) greatly reduced the relaxation caused by PAPS (Fig. 3b).

In order to test a possible antagonist effect of PAPS, three concentration–relaxation curves of ADPβS (acting through P2Y receptors), UTP (acting through P2U receptors) or acetylcholine (acting through muscarine receptors) were determined in each aortic ring (cf. Hansmann et al.,

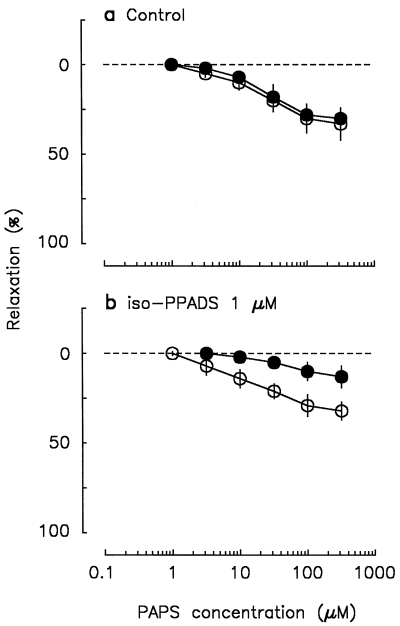

Fig. 3. Rat aorta: relaxation by PAPS and interaction with *iso*-PPADS. Noradrenaline (1 μM) was added to the medium twice, interval 60 min. PAPS was administered in a cumulative fashion during the plateau of each response to noradrenaline. Solvent (a) or *iso*-PPADS (1 μM; (b)) was added immediately after the first PAPS concentration–relaxation curve, i.e., about 50 min before the second concentration–relaxation curve. Abscissae show concentration of PAPS; ordinates show relaxation in first (○) and second (●) curves as a percentage of the respective response to noradrenaline. Means ± S.E.M. from four experiments.

1997). In the first curve, the EC_{50} (and maximal percentage relaxation) values were 0.48 ± 0.02 μM ($87 \pm 1\%$) for ADPβS ($n = 12$), 1.2 ± 0.2 μM ($70 \pm 4\%$) for UTP ($n = 10$) and 36 ± 2 nM ($97 \pm 1\%$) for acetylcholine ($n = 11$). For either agonist, a second and third curve, after addition of solvent (water), was close to the first ($n = 5$ to 7; not shown).

PAPS (10 or 100 μM), when added after completion of the first concentration–response curve of ADPβS, UTP or acetylcholine, did not change the tension of the (non-precontracted) aorta and also did not change subsequent contractions elicited by noradrenaline. PAPS (10 and 100 μM) shifted the concentration–relaxation curve of ADPβS to the right and progressively decreased the maximum (Fig. 4a; Table 1). PAPS (10 μM) did not alter the concentration–relaxation curves of UTP (Fig. 4b) and

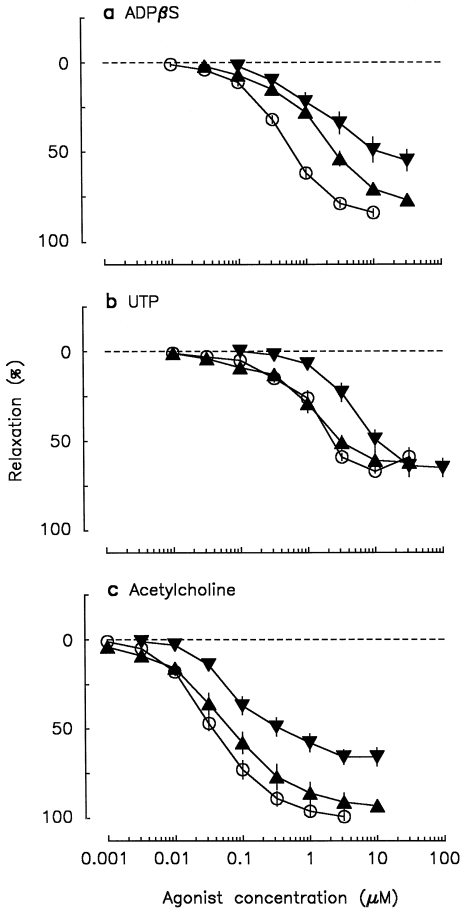

Fig. 4. Rat aorta: effect of PAPS on nucleotide- and acetylcholine-evoked relaxations. Noradrenaline (1 μM) was added to the medium three times, interval 60 min. ADPβS (a), UTP (b) or acetylcholine (c) was administered in a cumulative fashion during the plateau of each response to noradrenaline. PAPS was added at two increasing concentrations immediately after the first and second nucleotide or acetylcholine concentration–relaxation curve, i.e., about 50 min before the second and third concentration–relaxation curve. Abscissae show agonist concentration; ordinates show relaxation in first curves (○), and second and third curves in the presence of PAPS (10 μM ▲; 100 μM ▼), as a percentage of the respective response to noradrenaline. Means ± S.E.M. from four to seven experiments.

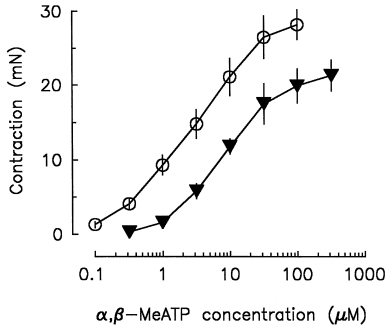

Fig. 5. Rat vas deferens: effect of PAPS on α,β-MeATP-evoked contractions. Increasing concentrations of α,β-MeATP were added every 15 min and washed out immediately after the contraction had peaked. Two concentration–response curves were determined in each tissue. Solvent (○) or PAPS (100 μM ▼) was added after completion of the first curve and the second curve was determined 120 min later. Abscissae, concentration of α,β-MeATP. Ordinates show contraction (mN) in second curves. Means ± S.E.M. from three to five experiments.

acetylcholine (Fig. 4c) but PAPS (100 μM) shifted either curve to the right and, in the case of acetylcholine, also decreased the maximum (Fig. 4b and c). As in the guinea-pig taenia coli, the concentration ratios indicate that PAPS did not differentiate well between UTP, acetylcholine and ADPβS (Table 1).

3.3. Contraction of rat vas deferens

PAPS (100 μM) elicited small, transient contractions of the rat vas deferens (0.6 ± 0.3 mN; $n = 3$); lower concentrations were without effect. Because of its small size, the response was not investigated further.

In order to test a possible antagonist effect of PAPS, two concentration–contraction curves of α,β-MeATP (acting through P2X receptors) and noradrenaline (acting through α_1-adrenoceptors) were determined in each vas deferens (cf. Bültmann and Starke, 1995). In the first curve, the EC_{50} value (and maximal contraction) was 2.9 ± 0.7 μM (24.9 ± 1.4 mN) for α,β-MeATP ($n = 12$) and 4.9 ± 0.8 μM (23.3 ± 0.9 mN) for noradrenaline ($n = 6$). For either agonist, a second curve, after addition of solvent (water), was close to the first ($n = 5$ and 3; not shown).

PAPS (10 μM) did not alter the concentration–contraction curve of α,β-MeATP ($n = 3$; not shown). PAPS (100 μM) shifted the curve to the right and decreased the maximum (Fig. 5a; Table 1). PAPS (100 μM) did not alter contractions elicited by noradrenaline ($n = 3$; not shown).

4. Discussion

4.1. Guinea-pig taenia coli

In the guinea-pig taenia coli, PAPS elicited prominent relaxation that was attenuated by the P2 receptor antago-

nists DIDS and reactive red 2 (Fig. 1). The apparent K_d value of DIDS against PAPS (0.27 μM) is close to its K_d against α,β-MeATP (0.7 μM) but much lower than its K_d against ADPβS in the taenia coli (70.1 μM; Dudeck et al., 1995). The apparent K_d of reactive red 2 against PAPS (0.29 μM) also is close to its K_d against α,β-MeATP (1.6 μM) in the taenia but, in this case, considerably higher than the K_d against ADPβS (0.028 μM; Bültmann and Starke, 1995). Apparently, PAPS activates the P2 receptor which is also the site of action of α,β-MeATP to cause relaxation of the taenia coli (cf. Bültmann and Starke, 1995; Dudeck et al., 1995; Windscheif et al., 1995; Bültmann et al., 1996). There is no evidence for an agonist action at the P2Y receptor, the site of action of ADPβS.

When tested as an antagonist, PAPS attenuated relaxations of the taenia coli caused by ADPβS (mediated by P2Y receptors), α,β-MeATP (mediated by the separate α,β-MeATP-receptors) or noradrenaline (mediated by α_1-adrenoceptors; Fig. 2; Table 1). PAPS, hence, displays little, if any, P2Y selectivity in the guinea-pig taenia coli. The noncompetitive (Fig. 2a; Table 1) inhibitory effect against ADPβS may result from P2Y receptor blockade (see Introduction) but may also be due to heterologous desensitization following activation of the separate α,β-MeATP-receptor. The apparent antagonism against α,β-MeATP and noradrenaline possibly results from homologous and heterologous desensitization, respectively. In fact, α,β-MeATP itself attenuates the hyperpolarization of the taenia coli produced by noradrenaline (Den Hertog and van den Akker, 1986).

4.2. Rat aorta

In rat aorta, PAPS elicited weak relaxation that was attenuated by the P2 antagonist *iso*-PPADS (Fig. 3). *iso*-PPADS, at a concentration of 1 μM, antagonizes the P2Y receptor-mediated relaxation of the rat aorta caused by ADPβS (and MeSATP) but does not alter the P2U receptor-mediated relaxation caused by UTP (and ATP; Hansmann et al., 1997). Apparently, PAPS activates the (endothelial) P2Y receptors to cause relaxation of rat aorta. Compared to ADPβS (Fig. 4a), PAPS behaves as a partial agonist at this receptor.

When tested as an antagonist, PAPS attenuated relaxations of the aorta caused by ADPβS (mediated by P2Y receptors), UTP (mediated by P2U receptors) or acetylcholine (mediated by muscarine receptors; Fig. 4; Table 1). PAPS, hence, displays little, if any, P2Y-selectivity in the rat aorta. The noncompetitive (Fig. 4a) effect against ADPβS is probably due to the partial agonist action of PAPS at the P2Y receptor, resulting in either blockade or homologous desensitization. The mechanism underlying the apparent antagonism against UTP and acetylcholine is uncertain. It is not due to heterologous desensitization, as preincubation with MeSATP, an agonist with high intrinsic activity at the P2Y receptor, does not alter the relaxation of the rat aorta caused by UTP (Dainty et al., 1991; Hansmann et al., 1997) and acetylcholine (G. Hansmann and R. Bültmann, unpublished observation).

4.3. Rat vas deferens

In rat vas deferens, PAPS elicited very small contractions. Its mechanism of action was not investigated in detail.

When tested as an antagonist, PAPS attenuated contractions of the vas deferens elicited by α,β-MeATP (mediated by P2X receptors; Fig. 5) but did not alter the response to noradrenaline (mediated by α_1-adrenoceptors). PAPS, hence, displays a certain degree of selectivity for the P2X receptor in rat vas deferens (Table 1). The noncompetitive (Fig. 5) effect against α,β-MeATP may result from blockade of the P2X receptor, but also from homologous desensitization if one assumes that the small contraction caused by PAPS was due to P2X receptor activation. As shown by the concentration ratios obtained in the presence of 100 μM PAPS (Table 1), PAPS was about as effective against α,β-MeATP in rat vas deferens as it was against relaxation-producing agonists in guinea-pig taenia coli and rat aorta.

5. Conclusion

Although evidence was obtained for a possible partial agonist or antagonist activity of PAPS at native P2Y receptors, the nucleotide is at best slightly selective for this subtype. Most prominently, it acts as an agonist at the P2 receptor activated by α,β-MeATP in the guinea-pig taenia coli. In addition, it blocks (or desensitizes) the P2X receptor of rat vas deferens and blocks non-P2 receptor effects in guinea-pig taenia coli and rat aorta. Taken together, these findings question the suitability of PAPS as a P2Y receptor antagonist in intact tissues.

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References

Boyer, J.L., Romero-Avila, T., Schachter, J.B., Harden, T.K., 1996. Identification of competitive antagonists of the $P2Y_1$ receptor. Mol. Pharmacol. 50, 1323–1329.

Bültmann, R., Starke, K., 1994. P2-purinoceptor antagonists discriminate three contraction-mediating receptors for ATP in rat vas deferens. Naunyn-Schmiedeberg's Arch. Pharmacol. 349, 74–80.

Bültmann, R., Starke, K., 1995. Reactive red 2: a P2Y-selective

purinoceptor antagonist and an inhibitor of ecto-nucleotidase. Naunyn-Schmiedeberg's Arch. Pharmacol. 352, 477–482.

Bültmann, R., Dudeck, O., Starke, K., 1996. Evaluation of P2-purinoceptor antagonists at two relaxation-mediating P2-purinoceptors in guinea-pig taenia coli. Naunyn-Schmiedeberg's Arch. Pharmacol. 353, 445–451.

Burnstock, G., Kennedy, C., 1985. Is there a basis for distinguishing two types of P2-purinoceptor?. Gen. Pharmacol. 16, 433–440.

Dainty, I.A., O'Connor, S.E., Leff, P., 1991. Endothelium-dependent relaxations to UTP in the rat aorta are not mediated by P2Y-purinoceptors. Fundam. Clin. Pharmacol. 5, 387.

Den Hertog, A., van den Akker, J., 1986. The influence of α,β-methylene ATP on α_1-receptor-operated channels in guinea-pig taenia caeci. Eur. J. Pharmacol. 122, 117–121.

Den Hertog, A., Pielkenrood, J., Ras, R., van den Akker, J., 1984. The contribution of calcium and potassium to the α-action of adrenaline on smooth muscle cells of the portal vein, pulmonary artery and taenia coli of the guinea pig. Eur. J. Pharmacol. 98, 223–234.

Dudeck, O., Bültmann, R., Starke, K., 1995. Two relaxation-mediating P2-purinoceptors in guinea-pig taenia coli. Naunyn-Schmiedeberg's Arch. Pharmacol. 351, 107–110.

Fredholm, B.B., Abbracchio, M.P., Burnstock, G., Daly, J.W., Harden, T.K., Jacobson, K.A., Leff, P., Williams, M., 1994. VI. Nomenclature and classification of purinoceptors. Pharmacol. Rev. 46, 143–156.

Fredholm, B.B., Abbrachio, 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.

Furchgott, R.F., 1972. The classification of adrenoceptors (adrenergic receptors): an evaluation from the standpoint of receptor theory. In: Blaschko, H., Muscholl, E. (Eds.), Catecholamines: Handbook of Experimental Pharmacology, Vol. 33. Springer, Berlin, pp. 283–335.

Hansmann, G., Bültmann, R., Tuluc, F., Starke, K., 1997. Characterization by antagonists of P2-receptors mediating endothelium-dependent relaxation in the rat aorta. Naunyn-Schmiedeberg's Arch. Pharmacol. 356, 641–652.

Harden, T.K., Lazarowski, E.R., Boucher, R.C., 1997. Release, metabolism and interconversion of adenine and uridine nucleotides: implications for G protein-coupled P2 receptor agonist selectivity. Trends Pharmacol. Sci. 18, 43–46.

Khakh, B.S., Michel, A., Humphrey, P.P.A., 1994. Estimates of antagonist affinities at P2X purinoceptors in rat vas deferens. Eur. J. Pharmacol. 263, 301–309.

Khakh, B.S., Surprenant, A., Humphrey, P.P.A., 1995. A study on P2X purinoceptors mediating the electrophysiological and contractile effects of purine nucleotides in rat vas deferens. Br. J. Pharmacol. 115, 177–185.

Lambrecht, G., Niebel, B., Ziyal, R., Ardanuy, U., Bäumert, H.G., Nickel, P., Mutschler, E., 1996. P2-purinoceptor heterogeneity in guinea-pig taenia coli and rabbit vas deferens. Naunyn-Schmiedeberg's Arch. Pharmacol. 353, R42.

Motulsky, H.J., Ransnas, L.A., 1987. Fitting curves to data using nonlinear regression: a practical and nonmathematical review. FASEB J. 1, 365–374.

O'Connor, S.E., Dainty, I.A., Leff, P., 1991. Further subclassification of ATP receptors based on agonist studies. Trends Pharmacol. Sci. 12, 137–141.

Pacaud, P., Feolde, E., Frelin, C., Loirand, G., 1996. Characterization of the P2Y-purinoceptor involved in the ATP-induced rise in cytosolic Ca^{2+} concentration in rat ileal myocytes. Br. J. Pharmacol. 118, 2213–2219.

Schachter, J.B., Qing, L., Boyer, J.L., Nicholas, R.A., Harden, T.K., 1996. Second-messenger cascade specificity and pharmacological selectivity of the human P_{2Y1}-purinoceptor. Br. J. Pharmacol. 118, 167–173.

Valera, S., Hussy, N., Evans, R.J., Adami, N., North, R.A., Surprenant, A., Buell, G., 1994. A new class of ligand-gated ion channel defined by P2X receptor for extracellular ATP. Nature 371, 516–519.

Waud, D.R., 1976. Analysis of dose–response relationships. In: Narahashi, T., Bianchi, C.P. (Eds.), Advances in General and Cellular Pharmacology, Vol. 1. Plenum, New York, pp. 145–178.

Windscheif, U., Pfaff, O., Ziganshin, A.U., Hoyle, C.H.V., Bäumert, H.G., Mutschler, E., Burnstock, G., Lambrecht, G., 1995. Inhibitory action of PPADS on relaxant responses to adenine nucleotides or electrical field stimulation in guinea-pig taenia coli and rat duodenum. Br. J. Pharmacol. 115, 1509–1517.