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ORIGINAL ARTICLE

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Reactive red 2: a P_{2Y}-selective purinoceptor antagonist and an inhibitor of ecto-nucleotidase

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Abstract Effects of reactive red 2 and its parent compound acid red 33 were studied in rat vas deferens and guinea-pig taenia coli. In rat vas deferens, reactive red 2 (1 to 10 μM) shifted the concentration-response curve for the P_{2X}-purinoceptor-mediated contraction effect of α, β-methylene ATP slightly to the right and progressively decreased the maximum (apparent antagonist K_d value 0.42 μM). Acid red 33 (1000 μM) shifted the curve to the right without changing the maximum (apparent K_d 386 μM). The concentration-contraction curve of noradrenaline was not altered by reactive red 2. In the carbachol-precontracted guinea-pig taenia coli, reactive red 2 (0.1 to 10 μM) shifted the concentration-response curve for the P_{2Y}-purinoceptor-mediated relaxation effect of adenosine 5′-O-(2-thiodiphosphate) (ADPβS) progressively to the right; only at the highest concentration of antagonist (10 μM) was the maximum slightly depressed; a pA_2 value of 7.55 (K_d 0.028 μM) was derived from the shift. Acid red 33 (1000 μM) shifted the concentration-relaxation curve of ADPβS to the right without changing the maximum (apparent K_d 171 μM). Reactive red 2 (1 to 10 μM) also shifted the concentration-response curve for the relaxation effect of α, β-methylene ATP, which is mediated by an unclassified P_2-purinoceptor, progressively to the right but simultaneously decreased the maximum (apparent K_d 1.6 μM). The concentration-relaxation curve of 2-chloroadenosine was not altered by reactive red 2. Pieces of vas deferens and taenia coli degraded 76 and 66 % of added ATP (10 μM) within 30 min, respectively. Reactive red 2 (0.1 to 100 μM) progressively reduced this degradation by up to 95%, with IC_{50}values of 3.9 ± 0.6 and 3.9 ± 2.3 μM, respectively. Acid red 33 (1000 μM) reduced the degradation by 30 and 20%, respectively.

The results indicate that reactive red 2 is a relatively potent antagonist at both P_{2X}-purinoceptors in rat vas deferens and P_{2Y}-purinoceptors in guinea-pig taenia coli, with a 15 fold selectivity for the P_{2Y}-purinoceptor. It inhibits ecto-nucleotidase in both tissues. The dichloro-triazine residue that distinguishes the compound from acid red 33 greatly enhances the potency at both receptor subtypes as well as at the nucleotidase. As regards P_2-purinoceptor subtypes, the results confirm the existence of two relaxation-mediating P_2-purinoceptors in guinea-pig taenia coli.

Key words Rat vas deferens · Guinea-pig taenia coli · Reactive red 2 · Acid red 33 · P_{2X}-Purinoceptor · P_{2Y}-Purinoceptor · Ecto-nucleotidase

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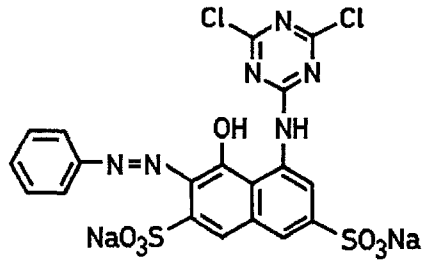
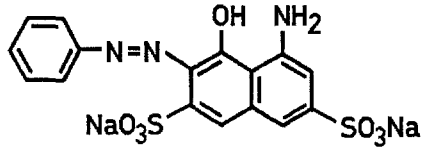
Introduction

There is a need of potent and subtype-selective P_2-purinoceptor antagonists. Several substances that display selectivity for the P_{2X} subtype have recently been described: pyridoxalphosphate-6-azophenyl-2′,4′-disulphonic acid (PPADS; Lambrecht et al., 1992; Windscheif et al., 1994) and its 2′,5′-disulphonic acid isomer (*iso*-PPADS; Bültmann et al. 1994a; Connolly et al, 1995), 4,4′-diisothiocyanatostilbene-2,2′-disulphonic acid (DIDS; Bültmann and Starke 1994a; Dudeck et al. 1995) and 8,8′-[carbonylbis(imino-3,1-phenylene) carbonylimino]bis-(1,3,5-naphthalenetrisulphonic acid) (NFO23; Ziyal et al. 1994). On the other hand, there is no selective antagonist for the P_{2Y} subtype. Reactive blue 2 and cibacron blue 3GA, formerly regarded as reasonably selective P_{2Y}-purinoceptor antagonists (see Kennedy 1990), block P_{2X}-purinoceptors with similar affinity (Bültmann and Starke 1994b; Khakh et al. 1994).

Reactive red 2 (Fig. 1) combines chemical features of two groups of P_2-purinoceptor antagonists, namely the "reactive dyes" reactive blue 2 and cibacron blue



Reactive red 2



Acid red 33

Fig. 1 Structures of reactive red 2 and acid red 33

3GA with which it shares the reactive chlorine-substituted triazine ring, and the azo dyes Evans blue and trypan blue (Bültmann and Starke 1993; Bültmann et al.1994b; Khakh et al. 1994). We have now investigated the effects of reactive red 2 and its parent compound acid red 33 (Fig. 1) on contractions of the rat vas deferens elicited by α, β-methylene ATP (α, β-MeATP; mediated by P_{2X}-purinoceptors; Bültmann and Starke 1994b; Khakh et al. 1994) and on relaxations of the carbachol-precontracted guinea-pig taenia coli elicited by adenosine 5′-O-(2-thiodiphosphate) (ADPβS; mediated by P_{2Y}-purinoceptors) and α, β-MeATP (mediated by a P_2-receptor distinct from P_{2Y}; Bültmann et al. 1994a; Windscheif et al. 1994; Dudeck et al. 1995).

Several P_2-purinoceptor antagonists block ecto-nucleotidases (Hourani and Chown 1989; Bültmann et al. 1994a, 1995; Crack et al. 1994; van Rhee et al. 1994). This blockade attenuates the apparent antagonism against substrates of the enzyme such as ATP (see Crack et al. 1994; Bültmann et al. 1995). Hence, the ecto-nucleotidase blocking properties of reactive red 2 and acid red 33 were determined as well.

Methods

Male Wistar rats (260 to 340 g) or guinea-pigs (480 to 800 g) were decapitated and the vasa deferentia (rat) or the ventral taenia coli (guinea-pig) removed and cleaned of adherent tissue. 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.

Contraction of rat vas deferens. Prostatic thirds of the vas deferens were suspended vertically in a 5.9 ml organ bath. The lower end was fixed and the upper end attached to an isometric force transducer (K30, Hugo Sachs Elektronik, Hugstetten, Germany). The initial tension applied was 9.8 mN (Graphtec thermal pen recorder, Ettlingen, Germany), but tissues subsequently relaxed to approximately 3 mN during a 60 min equilibration period. The medium was replaced every 15 min. Only one agonist (α, β-MeATP or noradrenaline) was tested on each preparation. Agonists were washed out after contractions had peaked. Maximum contraction amplitudes were measured. For concentration-response curves, increasing concentrations of agonist were added every 30 min.Two concentration-response curves were determined per preparation. Solvent or antagonists were added after completion of the first curve, and the second curve was determined 120 min later.

Relaxation of guinea-pig taenia coli. Strips of about 10 mm of the taenia coli were suspended vertically in a 5.9 ml organ bath. The lower end was fixed and the upper end attached to an isometric force transducer (K30). The medium was replaced every 15 min. The resting tension was adjusted to 9.8 mN during an initial 60 min equilibration period. Carbachol was then added to the medium at 15 min intervals for 2 to 3 min each. Initially, the maximal contraction of each strip was determined by a single addition of carbachol 300 nM. During the following 2 to 4 applications, the concentration of carbachol giving about 80% of the maximum was determined. This concentration (50 – 90 nM) was used for the remainder of the experiment, the 15 min rhythm being kept throughout. Agonists causing relaxation were added during the plateau of the carbachol response and washed out together with the latter after the relaxation was maximal. Only one agonist (ADPβS, α, β-MeATP or 2-chloroadenosine) was tested per taenia coli strip. Relaxations were expressed as a percentage of the respective carbachol contraction. Concentration-response curves were determined as in rat vas deferens.

Removal of ATP from medium. Vasa deferentia were split open longitudinally and pieces (9 mg average wet weight) were cut from the prostatic portion. Similarly, strips of about 10 mm (11 mg average wet weight) were cut from the taenia coli. Single tissue pieces were incubated for 120 min in 0.6 ml medium or medium containing inhibitor (reactive red 2 or acid red 33). ATP stock solution, 10 µl, was then added to give an initial concentration of 10 µM. Two aliquots of 100 µl were removed, the first immediately after addition of ATP, without replacement, the second 30 min later. Aliquots were diluted 1:100. ATP was assayed by means of luciferin-luciferase (see Driessen et al. 1993). Reactive red 2 and acid red 33 did not interfere with the bioluminescence assay. The percentage degradation of ATP was calculated as "100 * (1 - content of second aliquot/content of first aliquot)" and corrected for spontaneous loss in the absence of tissue.

Evaluation of concentration-response data. Logistic curves were fitted to weighted mean values by means of equation No. 25 of Waud (1976) and non-linear regression. The calculation yielded the maximal effect and the EC_{50} (agonists) or IC_{50} (inhibition of ATP breakdown), i.e. the concentration producing 50% of the maximum of that curve. Differences between fitted curves were tested according to p. 371 of Motulsky and Ransnas (1987). Apparent antagonist K_d values were derived as follows. When the maximum of the agonist concentration-response curve remained unaltered in the presence of the antagonist, the shift of the curve caused by the antagonist was read at the level of the EC_{50}, and the apparent K_d value calculated by means of equation no. 4 of Furchgott (1972) or by means of a plot according to Arunlakshana and Schild (1959). When, on the other hand, the maximum of the agonist concentration-response curve

was decreased in the presence of the antagonist, the apparent antagonist K_d value was derived using a double-reciprocal plot according to p. 335 of Kenakin (1993).

Materials. Reactive red 2 was synthesized in our laboratory from acid red 33 (gift from Dr. C. Dudeck, BASF, Ludwigshafen, Germany) and cyanuric chloride (Aldrich, Steinheim, Germany). The identity and purity of the product was confirmed by TLC and ^{1}H-NMR spectroscopy. TLC was performed on silica gel F_{254} (Merck, Darmstadt, Germany) with ethylacetate/methanol (2:1, v/v) as eluent. The R_f values were 0.18 for acid red 33 and 0.21 for reactive red 2. The ^{1}H-NMR spectrum of reactive red 2 was obtained on a Bruker 400 MHz-spectrometer using dimethylsulfoxide-d_6 as solvent. The peaks were assigned as follows: H2 (d, 8.85), H4 (s, 7.68), H5/H3′/H5′ (three proton multiplett, 7.50), H2′/H6′ (d, 7.93), H4′ (t, 7.30). Other drugs used were adenosine 5′-O-(2-thiodiphosphate) trilithium (ADPβS), α,β-methylene ATP dilithium (α,β-MeATP), ATP, 2-chloroadenosine and (−)-noradrenaline bi-(+)-tartrate (Sigma, Deisenhofen, Germany). Stock solutions were made up in distilled water. Drug solutions were added to the organ bath in aliquots not exceeding 100 µl.

Statistics. Data are expressed as either the arithmetic mean ± SEM or, in the case of fitted curves, the EC_{50} and maximal effect with the SE as defined by Waud (1976). $P < 0.05$ was taken as the limit of statistical significance.

Results

Contraction of rat vas deferens

Increasing concentrations of α, β-MeATP elicited increasing contraction (EC_{50} 3.4 ± 0.3 µM, maximal contraction 24.6 ± 0.5 mN; $n = 25$; first concentration-contraction curves). A second concentration-contraction curve after addition of solvent was similar to the first although the maximum was increased (open circles in Fig. 2). Reactive red 2 (0.32 µM) caused no change (Fig. 2). Reactive red 2 (1 µM) shifted the curve slightly to the right ($P < 0.05$) and decreased the maximum (by 19 %; $P < 0.05$; Fig. 2). An apparent antagonist K_d value of 0.42 µM was derived from the effect of reactive red 2 (1 µM). Reactive red 2 (3.2 and 10 µM) markedly depressed contractions over the entire range of α, β-MeATP concentrations studied (Fig. 2).

Acid red 33 (1000 µM) shifted the concentration-response curve of α, β-MeATP to the right (EC_{50} 17.3 ± 2.1 µM) without changing the maximum ($n = 4$). An apparent K_d of 386 µM was calculated from the shift.

Increasing concentrations of noradrenaline (0.1 to 1000 µM) likewise elicited increasing contraction (EC_{50} 16.4 ± 3.2 µM, maximal contraction 23.4 ± 1.0 mN; $n = 9$; first concentration-response curves). A second concentration-response curve after addition of solvent was similar to the first (EC_{50} 10.4 ± 3.1 µM, maximal contraction 26.1 ± 1.6 mN; $n = 4$). Reactive red 2 (10 µM) did not alter the concentration-contraction curve of noradrenaline (EC_{50} 7.7 ± 2.0 µM, maximal contraction 33.2 ± 1.8 mN; $n = 5$; $P > 0.05$).

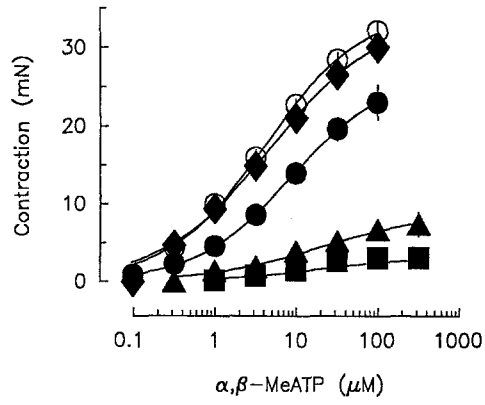


Fig. 2 Effect of reactive red 2 on the concentration-response curve of α, β-methylene ATP (α, β-MeATP) in rat vas deferens. Increasing concentrations of α, β-MeATP were added every 30 min. Two concentration-response curves were determined in each tissue. Solvent (○) or reactive red 2 (0.32 µM ◆; 1 µM ●; 3.2 µM ▲; 10 µM ■) was added to the medium after completion of the first curve and the second curve was determined 120 min later. *Ordinates* show contraction in second concentration-response curves. Curves are the fitted sigmoids. Means ± SEM from 4 to 5 experiments.

The reversibility of the effect of reactive red 2 and acid red 33 was examined in separate experiments. α,β-MeATP (3.2 µM) was administered every 60 min (first response 10.3 ± 0.6 mN, $n = 13$; all experiments pooled). Solvent or the antagonist to be tested was added after the first and during the second response to α, β-MeATP and then washed out. Contractions to α,β-MeATP increased in solvent controls (by 37 ± 13 % at 5th addition, $n = 6$). Reactive red 2 (10 µM) and acid red 33 (1000 µM), while present in the medium (second response to α, β-MeATP), reduced the contraction by 88 ± 6 and 83 ± 2%, respectively. The antagonism by reactive red 2 was attenuated to a 42 ± 9%, 35 ± 13% and 31 ± 13% reduction after washout for 60, 120 and 180 min ($n = 4$; all corrected for the solvent controls). The antagonism by acid red 33, in contrast, disappeared after washout for 60 min ($n = 3$).

Reactive red (up to 10 µM) did not change the resting tension of the rat vas deferens.

Relaxation of guinea-pig taenia coli

When added during the plateau of the contraction elicited by carbachol (50 to 90 nM; force of contraction 83 mN on average), increasing concentrations of ADPβS and α, β-MeATP caused increasing relaxation (EC_{50} values 63 ± 3 nM and 2.2 ± 0.2 µM, maximal relaxations 98 ± 2 and 101 ± 2%, respectively; $n = 46$ and 24; first concentration-relaxation curves). A second concentration-relaxation curve, after addition of solvent, was similar to the first one for either agonist (open circles in Fig. 3A and B).

Increasing concentrations of reactive red 2 (0.1 to 10 μM) shifted the concentration-relaxation curve of ADPβS progressively to the right (Fig. 3A). The curve became steeper in the presence of the antagonist ($P < 0.05$ for reactive red 2 0.32 to 10 μM). At the highest concentration of reactive red 2 (10 μM), the maximum of the curve was slightly decreased ($P < 0.05$). A plot according to Arunlakshana and Schild (1959) of the data obtained with 0.1 to 3.2 μM of reactive red 2 was linear, slope 1.10 ± 0.08 ($P > 0.05$ versus unity), and yielded a pA_2 value of 7.55 (K_d 28 nM).

The concentration-response curve of α, β-MeATP was not altered by reactive red 2 (0.32 μM; Fig. 3B). Reactive red 2 (1 to 10 μM) shifted the curve to the right

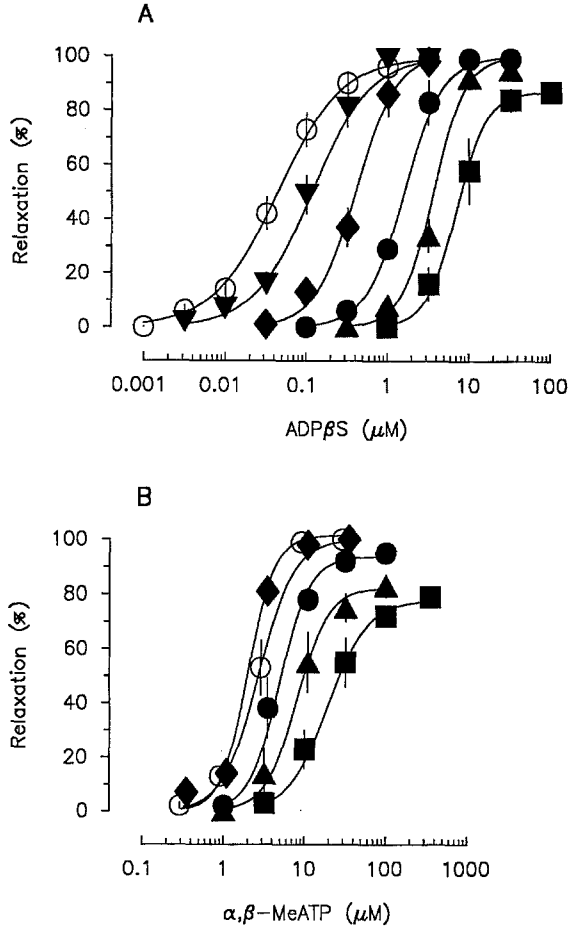


Fig. 3 Effect of reactive red 2 on concentration-response curves of α,β-methylene ATP (α, β-MeATP) and adenosine 5'-O-(2-thiodiphosphate) (ADPβS) in carbachol-precontracted guinea-pig taenia coli. Increasing concentrations of ADPβS (**A**) or α, β-MeATP (**B**) were added with each successive carbachol dose, i.e. every 15 min. Two concentration-response curves were determined in each tissue. Solvent (○) or reactive red 2 (0.1 μM ▼; 0.32 μM ◆; 1 μM ●; 3.2 μM ▲; 10 μM ■) was added to the medium after completion of the first curve and the second curve was determined 120 min later. *Ordinates* show relaxation in second concentration-response curves as a percentage of the respective response to carbachol. Curves are the fitted sigmoids. Means ± SEM from 5 to 8 experiments

and decreased the maximum ($P < 0.05$; Fig. 3B). An apparent antagonist K_d value of 1.6 μM was derived from the effect of reactive red 2 (3.2 μM).

Acid red 33 (1000 μM) shifted the concentration-relaxation curve of ADPβS to the right (EC_{50} 210 ± 17 nM) without changing the maximum ($n = 5$). An apparent K_d of 171 μM was calculated from the shift.

Increasing concentrations of 2-chloroadenosine (1 to 320 μM) likewise caused increasing relaxation of the carbachol-precontracted guinea-pig taenia coli (EC_{50} 17.5 ± 2.5 μM, maximal relaxation 101 ± 3%, $n = 13$; first concentration-response curves). A second curve, after addition of solvent, was similar to the first (EC_{50} 19.1 ± 3.1 μM, maximal relaxation 100 ± 3%, $n = 6$). Reactive red 2 (10 μM) did not alter the concentration-response curve of 2-chloroadenosine (EC_{50} 30.4 ± 6.9 μM, maximal relaxation 101 ± 4%, $n = 7$; $P > 0.05$).

The reversibility of the effect of reactive red 2 and acid red 33 against ADPβS was again examined. ADPβS (0.1 μM) was administered every 60 min (first response 72 ± 4% relaxation, $n = 16$; all experiments pooled). Solvent or the antagonist to be tested was added after the first and during the second response to ADPβS and then washed out. Relaxations caused by ADPβS remained constant in solvent controls ($n = 6$). Reactive red 2 (0.32 μM) and acid red 33 (1000 μM), while present in the medium (second response to ADPβS), reduced the relaxation by 76 ± 4, and 81 ± 5%, respectively. The antagonism by reactive red 2 was attenuated to a 45 ± 4%, 40 ± 5% and 29 ± 6% reduction, the antagonism by acid red 33 to a 36 ± 5%, 30 ± 6% and 30 ± 6% reduction, after washout for 60, 120 and 180 min, respectively ($n = 5$ each; all corrected for the solvent controls).

Reactive red 2 (up to 10 μM) did not alter the spontaneous activity of the taenia or the contraction to carbachol.

Removal of ATP from medium

There was a spontaneous loss of 23 ± 2% of added ATP (10 μM) from the medium after 30 min of incubation in the absence of tissue ($n = 23$). Pieces of rat vas deferens or guinea-pig taenia coli degraded 76.4 ± 2.1 and 66.1 ± 2.7%, respectively, of added ATP within 30 min ($n = 14$ and 15). Increasing concentrations of reactive red 2 (0.1 to 100 μM) progressively reduced the removal of ATP in both tissues (Fig. 4). The IC_{50} values were 3.9 ± 0.6 and 3.9 ± 2.3 μM in rat vas deferens and guinea-pig taenia coli, respectively. Acid red 33 (1000 μM) reduced the removal of ATP in both tissues only slightly, to 69.6 ± 9.5% of control in the rat vas deferens ($n = 5$) and to 80.7 ± 6.7% of control in the guinea-pig taenia coli ($n = 7$). Reactive red 2 (100 μM) and acid red 33 (1000 μM) did not change the spontaneous loss of ATP ($n = 4$ each).

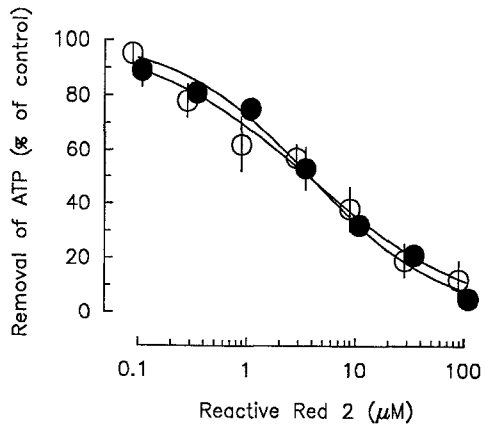


Fig. 4 Effect of reactive red 2 on the degradation of ATP in rat vas deferens and guinea-pig taenia coli. ATP, initial concentration 10 μM, was incubated with pieces of vas deferens (●) or taenia coli (○) for 30 min. *Ordinates* show removal of ATP within 30 min in the presence of reactive red 2 as a percentage of the removal obtained in solvent controls. Curves are the fitted sigmoids. Means ± SEM from 4 – 10 experiments

In some experiments, vas deferens or taenia coli pieces were incubated in medium for 120 min as in the experiments of Fig. 4, but the tissue was then removed from the medium before ATP (10 μM) was added for 30 min. Under these conditions, no degradation of ATP in excess of the spontaneous loss was obtained ($n = 4$ for vas deferens and taenia coli). The degradation of ATP in the presence of tissue was, therefore, not due to secreted enzymes or enzymes escaped from damaged cells.

Discussion

Reactive red 2 antagonized P_2-purinoceptor-mediated responses in rat vas deferens and guinea-pig taenia coli. Contraction of the rat vas deferens elicited by noradrenaline, contraction of the guinea-pig taenia coli elicited by carbachol, and relaxation of the taenia coli caused by 2-chloroadenosine remained unaltered, so the antagonism appears to be specific for P_2-purinoceptors.

Contraction of the rat vas deferens elicited by α,β-MeATP is mediated by P_{2X}-purinoceptors (Bültmann and Starke 1994b; Khakh et al. 1994). Reactive red 2 reduced these contractions in a non-competitive manner (Fig. 2). Like DIDS (cf. Fig. 2 of Bültmann and Starke 1994a), it shifted the concentration-response curve of α, β-MeATP only slightly to the right but greatly depressed the maximum. The antagonism by reactive red 2 was only partly reversible upon washout, again like the antagonism by DIDS (Bültmann and Starke 1994a). This non-competitve type of antagonism may reflect a partially irreversible blockade of the P_{2X}-purinoceptor due to covalent attachment by means of the reactive dichloro-triazine group of reactive red 2. In support of this view, acid red 33, which lacks the alkylating residue, shifted the concentration-response curve of α, β-MeATP to the right without changing the maximum, and the antagonism was fully reversible upon washout. Reactive red 2 is among the most potent antagonists at the P_{2X}-purinoceptor of rat vas deferens. The apparent K_d of 0.42 μM is similar to that of DIDS (2.5 μM; estimated from data shown in Fig. 2 of Bültmann and Starke 1994a), *iso*-PPADS (0.3 μM; Khakh et al. 1994), cibacron blue 3GA (1.6 μM; Khakh et al. 1994) and Evans blue (1.0 μM; Bültmann and Starke 1993).

Relaxation of the guinea-pig taenia coli caused by ADPβS is mediated by P_{2Y}-purinoceptors (Bültmann et al. 1994a; Dudeck et al. 1995). Except for a steepening of the concentration-response curve in the presence of the antagonist, reactive red 2 up to a concentration of 3.2 μM antagonized these relaxations in an apparently competitive manner, in contrast to rat vas deferens (Fig. 3A). However, as in rat vas deferens, the antagonism by reactive red 2 was only partly reversible upon washout and the lack of reversibility also held true for acid red 33, indicating that it was not due to a covalent attachment of reactive red 2 to the P_{2Y}-purinoceptor. Possibly, both antagonists bound to non-purinoceptor sites in the taenia coli, thus forming a reservoir that maintained some blockade of the P_{2Y}-purinoceptor during washout. Reactive red 2 is the most potent antagonist at the P_{2Y}-purinoceptor of the guinea-pig taenia coli described so far: with a K_d of 28 nM it is at least 350 times more potent than reactive blue 2 (16.7 μM) and suramin (9.7 μM; Bültmann et al. 1994a). Moreover, it displays selectivity, albeit only 15-fold, for the P_{2Y}-receptor in the guinea-pig taenia coli over the P_{2X}-receptor in rat vas deferens.

Relaxation of the guinea-pig taenia coli caused by α,β-MeATP, finally, has been suggested to be mediated by a second P_2-purinoceptor distinct from P_{2Y} (see Introduction). Reactive red 2 antagonized these relaxations in a non-competitive manner (Fig. 3B). Reactive red 2 was considerably less potent against α, β-MeATP (apparent antagonist K_d value 1.6 μM) than against ADPβS (K_d value 28 nM). The difference in potency, together with the different type of antagonism (Fig. 3), confirms the existence of two distinct sites of action for the two agonists. Among the antagonists that discriminate between ADPβS and α, β-MeATP in guinea-pig taenia coli (PPADS, XAMR0721 and DIDS; Windscheif et al. 1994; Bültmann et al. 1994a; Dudeck et al. 1995), only reactive red 2 displays higher affinity for the P_{2Y}-receptor than for the receptor activated by α,β-MeATP.

Reactive red 2 attenuated the removal, presumably by ecto-nucleotidase-catalyzed breakdown, of ATP from the medium by pieces of rat vas deferens or guinea-pig taenia coli (Fig. 4). Hence, like several other P_2-purinoceptor antagonists (see Introduction), it inhibits ecto-nucleotidases. This property greatly limits

its value as an antagonist to study responses caused by degradable agonists such as ATP. In comparison to other P_2-purinoceptor antagonists, reactive red 2 is a relatively potent inhibitor of ecto-nucleotidase. At a concentration of 100 μM only Evans blue caused a comparable degree of inhibition (Bültmann et al. 1995), while other antagonists such as suramin, PPADS and DIDS were less effective (R. Bültmann, unpublished data).

Whenever reactive red 2 was compared with the parent compound acid red 33, the former was considerably more potent than the latter. In the case of P_{2X}-purinoceptor antagonism (non-competitive), this is presumably due to the ability of the reactive dichloro-triazine to alkylate the receptor. In the case of P_{2Y}-purinoceptor antagonism (competitive), the dichloro-triazine residue presumably increases the affinity of the antagonist. The introduction of a dichloro-triazine residue may help to develop more potentP_2-purinoceptor antagonists and blockers of ecto-nucleotidase.

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