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P2X receptors counteract the vasodilatory effects of endothelium derived hyperpolarising factor

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Abstract

Dilatory responses of extracellular nucleotides were examined in the precontracted isolated rat mesenteric artery. Dilatation mediated by endothelium-derived hyperpolarising factor (EDHF) was studied in the presence of *N*ω-nitro-L-arginine (L-NOARG) and indomethacin, and was most potently induced by the selective $P2Y_1$ receptor agonist adenosine 5′-*O*-thiodiphosphate (ADPβS), while 2-methylthioadenosine triphosphate (2-MeSATP) and adenosine triphosphate (ATP) were almost inactive. However, after P2X receptor desensitisation (with αβ-methylene-adenosine triphosphate, αβ-MeATP), 2-MeSATP and ATP potently stimulated EDHF-mediated dilatation. This can be explained by simultaneous activation of endothelial P2Y receptors that release EDHF, and depolarising P2X receptors on smooth muscle cells. Uridine triphosphate (UTP) also induced potent dilatation, suggesting EDHF release via $P2Y_2/P2Y_4$ receptors. Uridine diphosphate (UDP) had only minor dilatory effects, and when pretreated with hexokinase it was almost inactive, suggesting a minor role for $P2Y_6$ receptors. The nitric oxide (NO) mediated dilatation was studied in the presence of charybdotoxin, apamin and indomethacin. ADPβS, 2-MeSATP, ATP and UTP were all potent relaxant agonists suggesting NO release via $P2Y_1$ and $P2Y_2/P2Y_4$ receptors, while UDP was much less potent and efficacious. P2X receptor desensitisation had only minor effect on the NO-mediated dilatations. In conclusion, both EDHF and NO-mediated dilatation can be induced by activation of $P2Y_1$ and $P2Y_2/P2Y_4$ receptors. P2X receptor stimulation of smooth muscle cells selectively counteracts the dilatory effect of EDHF.

Keywords: EDHF (endothelin-derived hyperpolarising factor); Endothelium; P2 receptor; Purine; Pyrimidine; Vasodilatation

1. Introduction

Purines and pyrimidines can be released from sympathetic nerves, platelets, endothelial cells and from most cell types when damaged (Goez et al., 1971; Gordon, 1986; Seifert and Schultz, 1989). They can induce both dilatation and contraction of blood vessels from most regions via activation of P2 receptors (Ralevic and Burnstock, 1998). Recent receptor cloning has proven the existence of several different P2X and P2Y receptor subtypes and their expression in cells has enabled characterisation of their pharmacological profile. The $P2X_1$ receptor is the dominating vascular P2X receptor subtype and activated by αβ-methylene-adenosine triphosphate (αβ-MeATP) > adenosine triphosphate (ATP) = 2-methylthioadenosine triphosphate (2-MeSATP) (Evans and Kennedy, 1994; Valera et al., 1994; Vulchanova et al., 1996). There are evidence that at least four P2Y receptor subtypes may mediate vascular effects, namely $P2Y_1$, $P2Y_2$, $P2Y_4$ and $P2Y_6$ (Harden et al., 1998). At the $P2Y_1$ receptor adenosine 5′-*O*-thiodiphosphate (ADPβS), 2-MeSATP and adenosine diphosphate (ADP) have greater potency than ATP, while uridine diphosphate (UDP) and uridine triphosphate (UTP) are inactive (Léon et al., 1997). The $P2Y_2$ and the rat $P2Y_4$ receptors are activated with similar potencies by UTP and ATP but not by UDP or ADP, while the $P2Y_6$ receptor is activated most potently by UDP and weakly by UTP, ADP and ATP (Nicholas et al., 1996; Webb et al., 1998).

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Generally, P2X and P2Y receptors on smooth muscle cells are contractile while P2Y receptors on endothelial cells induce dilatation mediated by release of nitric oxide (NO) and prostaglandins. In 1988, Taylor and Weston suggested that endothelium dependent dilatation of blood vessels to acetylcholine reflected the release of an additional factor, which could induce dilatation by increasing the membrane potential of smooth muscle cells (Taylor and Weston, 1988). This additional factor was termed endothelium-derived hyperpolarising factor (EDHF) (Chen et al., 1988). Although the identity of EDHF is still unknown, both its vascular dilatory and hyperpolarising properties can be successfully blocked by a combination of the K^+ channel inhibitors charybdotoxin and apamin (Corriu et al., 1996; Zygmunt and Högestätt, 1996; Zygmunt et al., 1998; White and Heily, 1997; Chataigneau et al., 1998).

Purines and pyrimidines have additionally been shown to dilate by an NO and prostaglandin independent pathway (Saïag et al., 1996; You et al., 1997) that can be antagonised by charybdotoxin and apamin, indicating involvement of EDHF (Malmsjö et al., 1998). However, to fulfil the criteria for EDHF release, hyperpolarisation of smooth muscle cells needs to be demonstrated. In a recent electrophysiological study we found that stimulation of endothelial P2Y receptors induced hyperpolarisation of smooth muscle cells, confirming that P2Y receptors mediate EDHF release (Malmsjö et al., 1999). Furthermore, we found that this effect was counteracted by the depolarising effect of simultaneous activation of P2X receptors (Malmsjö et al., 1999). The present study was designed to pharmacologically characterise the P2Y receptor subtypes that stimulate EDHF and NO-mediated dilatation using the recent knowledge of agonist potencies received from receptor cloning and to examine the effect of P2X receptor activation on EDHF-mediated dilatation.

2. Material and methods

2.1. Tissue preparation

Female Sprague–Dawley rats were used, as EDHF is functionally more prominent in female as compared to male rats (McCulloch and Randall, 1998). The rats (200 g) were anaesthetised by inhalation of CO_2 (carbon dioxide), after which they were killed by a cardiac cut. The mesenteric artery was removed gently and immersed in cold oxygenated buffer solution (for composition, see below) and dissected free from adhering tissue under a microscope. In experiments where endothelium denudation was required this was performed by perfusion of the vessel for 5 s with 0.1% Triton X followed by another 5 s of perfusion with a physiologic buffer solution (for composition, see below) using a fine needle. The vessels were cut into cylindrical segments (2–3 mm long) that were immediately used in the experiments. Each segment was mounted on two L-shaped metal prongs, one of which was connected to a force displacement transducer (FT03C) for continuous recording of the isometric tension, and the other to a displacement device. The position of the holder could be changed by means of a movable unit allowing fine adjustments of the vascular resting tension by varying the distance between the metal prongs. The mounted specimens were immersed in temperature controlled (37°C) tissue baths containing buffer solution of the following composition (mM): NaCl 119, $NaHCO_3$ 15, KCl 4.6, $MgCl_2$ 1.2, NaH_2PO_4 1.2, $CaCl_2$ 1.5 and glucose 5.5. The solution was continuously gassed with 5% CO_2 in O_2 giving a pH of 7.4. Twelve vessel segments were studied at the same time in separate tissue baths. These were allowed to stabilise at a resting tension of 2 mN for 1 h before the experiments were started. The contractile capacity of each segment was examined by exposure to a K^+ rich (60 mM) buffer solution in which NaCl was exchanged for an equimolar concentration of KCl. When two reproducible contractions had been achieved the vessels were used for further studies. Special care was taken to ensure that the endothelium was not damaged. In vessel segments perfused with Triton X, abolished dilatation to acetylcholine indicated a properly removed endothelium, while unaffected 3-morpholino-synonimine (SIN-1) dilatations suggested intact smooth muscle cell responsiveness to NO.

2.2. Measurement of dilatory responses

Dilatory responses to cumulatively added ADPβS, 2-MeSATP, ATP, UTP and UDP were studied in arteries with intact endothelium, precontracted with 10^{-6} M noradrenaline. To avoid desensitisation of receptors, only one series of each agonist in increasing concentrations was added to each segment. Antagonists were added 30 min before respective dilatory experiment. We have previously shown that indomethacin only slightly attenuated the ATP dilatation (approximately 10%) in the presence of *N*-nitro-L-arginine methyl ester (L-NAME), indicating a minor contribution of prostaglandins in this preparation (Malmsjö et al., 1998). All experiments were therefore performed in the presence of indomethacin (10^{-5} M), and the remaining dilatation was termed ''total dilatation''.

The *EDHF-mediated dilatation* was studied in the presence of indomethacin and *N*ω-nitro-L-arginine (L-NOARG, $10^{-3.5}$ M), in order to eliminate prostaglandin and NO-mediated effects, respectively. Arginine analogues like L-NOARG inhibit NO synthesis, but do not affect EDHF (Huang et al., 1988; Chen et al., 1991; Fujii et al., 1992). Although problems to completely inhibit NO synthase have been reported, the high concentration of L-NOARG used in the present experiments has been shown to totally block the release of NO (Zygmunt et al., 1994). This was also shown in our preparation by the lack of further

Table 1
Dilatory responses to ADPβS, 2-MeSATP, ATP, UTP, UDP and UDP + hexokinase (HK) in the isolated mesenteric arteries pretreated with either indomethacin and L-NOARG ($10^{-3,5}$ M) (*EDHF-dilatation*); indomethacin, charybdotoxin ($10^{-7.5}$ M) and apamin (10^{-6} M) (*NO-dilatation*); or indomethacin (10^{-5} M) (*total dilatation*).''P2X-des'' indicates that these agonists were added after P2X receptor desensitisation with 10^{-5} M αβ-MeATP. The responses are expressed as percentage of an initial contraction induced by 10^{-6} M noradrenaline. Data are shown as $E_{max} \pm$ S.E.M. (%) and $pEC_{50} \pm$ S.E.M. (log *M*) of eight experiments

	EDHF-dilatation: indomethacin + L-NOARG		*NO-dilatation*: indomethacin + charybdotoxin + apamin		*Total dilatation*: indomethacin	
	E_{max}	pEC_{50}	E_{max}	pEC_{50}	E_{max}	pEC_{50}
ADPβS	65 ± 5	6.1 ± 0.1	79 ± 6	6.0 ± 0.1	82 ± 4	6.5 ± 0.1
ADPβS (P2X-des)	69 ± 3	6.0 ± 0.1	78 ± 11	5.9 ± 0.1		
2-MeSATP	9 ± 2	4.9 ± 0.1	60 ± 9	6.2 ± 0.1	72 ± 8	5.9 ± 0.1
2-MeSATP (P2X-des)	51 ± 6	5.0 ± 0.2	72 ± 7	6.0 ± 0.1		
ATP	14 ± 4	4.4 ± 0.1	53 ± 11	5.7 ± 0.2	85 ± 4	5.9 ± 0.2
ATP (P2X-des)	69 ± 5	4.2 ± 0.1	75 ± 12	5.5 ± 0.2		
UTP	67 ± 6	5.1 ± 0.1	68 ± 10	5.9 ± 0.1	66 ± 4	6.1 ± 0.2
UTP (P2X-des)	60 ± 5	5.0 ± 0.2	70 ± 10	5.9 ± 0.1		
UDP	29 ± 4	4.2 ± 0.1	28 ± 5	4.3 ± 0.1	32 ± 8	4.2 ± 0.1
UDP + HK	8 ± 2	3.9 ± 0.1	20 ± 5	4.4 ± 0.2		

inhibitory effect by the NO scavenger, 2-phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (PTIO) (data not shown).

The *NO-mediated dilatation* was studied in the presence of indomethacin, charybdotoxin ($10^{-7.5}$ M) and apamin (10^{-6} M), in order to eliminate prostaglandin and EDHF-mediated effects, respectively. A combination of charybdotoxin and apamin has previously been shown to inhibit EDHF in the mesenteric artery (White and Heily, 1997; Chataigneau et al., 1998). Since the effect of dilators acting independently of the endothelium, such as SIN-1 or papaverin, was not affected, charybdotoxin plus apamin has proven their specificity against EDHF-mediated dilatation (Malmsjö et al., 1998).

To block all dilatory mediators, a combination of indomethacin, L-NOARG, charybdotoxin and apamin was used.

To distinguish between P2Y and P2X receptor effects, desensitisation of P2X receptors was performed by adding 10^{-5} M αβ-MeATP before each experiment (8 min), see results (Kasakov and Burnstock, 1983). To evaluate the action of the αβ-MeATP induced contraction on the following dilatory experiment, αβ-MeATP was substituted for a short noradrenaline contraction (10^{-7} M). To mimic the transient contraction elicited by αβ-MeATP, noradrenaline was added 8 min before each experiment and washed away after 1 min of contraction.

2.3. Drugs

Agonist purity and stability provides potential problems when delineating the pharmacological selectivity of P2 receptors especially in intact tissues. To avoid phosphorylation, hexokinase and glucose were used to convert UTP to UDP (Nicholas et al., 1996). Stock solutions of UDP in a concentration of 10^{-1} M was preincubated for 1 h with 10 units/ml hexokinase and 22 mN glucose. Metabolism of nucleotides was also prevented by use of ADPβS, which includes a thio substitution at the terminal phos-

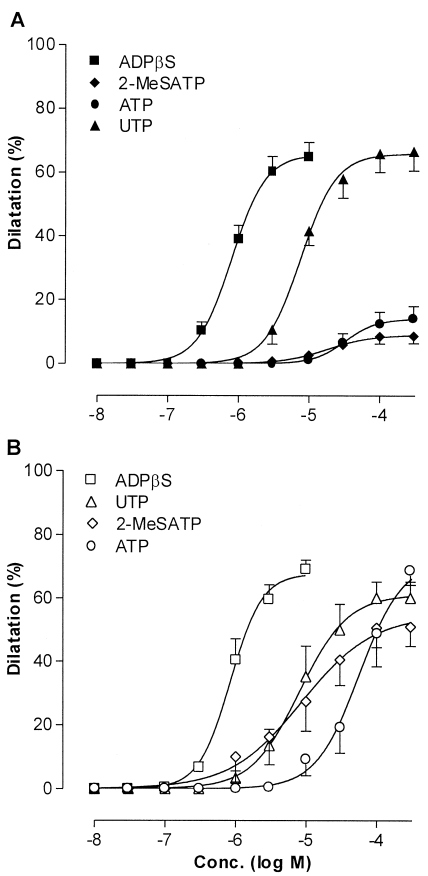

Fig. 1. *EDHF-mediated dilatations* were examined in the isolated mesenteric artery in the presence of indomethacin (10^{-5} M) and L-NOARG ($10^{-3.5}$ M). After precontraction with 10^{-6} M noradrenaline, dilatory concentration–response curves for ADPβS, 2-MeSATP, ATP, UTP were constructed without (A) and with prior P2X receptor desensitisation (10^{-5} M αβ-MeATP) (B). Data are shown as mean ± S.E.M. of eight experiments.

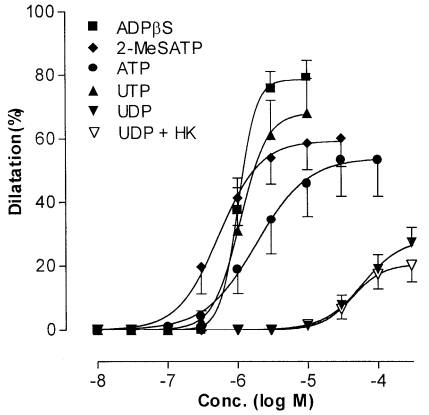

Fig. 2. *NO-mediated dilatations* were examined in the isolated mesenteric artery in the presence of indomethacin (10^{-5} M), charybdotoxin ($10^{-7.5}$ M) and apamin (10^{-6} M). After precontraction with 10^{-6} M noradrenaline, dilatory concentration–response curves for ADPβS, 2-MeSATP, ATP, UTP, UDP and UDP + hexokinase (HK) were constructed. Data are shown as mean ± S.E.M. of eight experiments.

phate that provides stability to ectonucleotidase action (Jacobson et al., 1998).

Acetylcholine, apamin (10^{-6} M), ATP, ADPβS, charybdotoxin ($10^{-7.5}$ M), hexokinase, indomethacin (10^{-5} M), L-NOARG ($10^{-3.5}$ M), noradrenaline, UTP, UDP, αβ-MeATP (10^{-5} M) and 2-MeSATP were purchased from Sigma, USA. All drugs were dissolved in 0.9% saline, except for hexokinase, which was dissolved in PBS.

2.4. Calculations and statistics

The amplitude of contraction before application of agonists was set to 100%. The negative logarithm of the drug concentration eliciting 50% dilatation (pEC_{50}) was determined by linear regression analysis using the values immediately above and below half-maximum response. E_{max} refers to maximum dilatation. Values are presented as mean ± S.E.M. All experiments were performed on eight segments (animals). Statistical significance was accepted when $P < 0.05$, using Students *t*-test. All differences referred to in the text have been statistically verified.

3. Results

Dilatory responses were examined in segments of the isolated rat mesenteric artery. The contractile response to 60 mM K^+ amounted to 5.0 ± 0.2 mN.

Dilatations induced by acetylcholine, ADPβS, 2-MeSATP, ATP, UTP, UDP or UDP were abolished by endothelium removal. The endothelium independent dilator, SIN-1 induced concentration dependent relaxant responses that did not differ significantly between control ($E_{max} = 101 \pm 7\%$ and $pEC_{50} = 5.1 \pm 0.1$) and endothelium denuded vessel segments (105 ± 4% and 5.2 ± 0.1).

P2X receptor desensitisation was performed by adding 10^{-5} M αβ-MeATP. This dose induced a transient con-

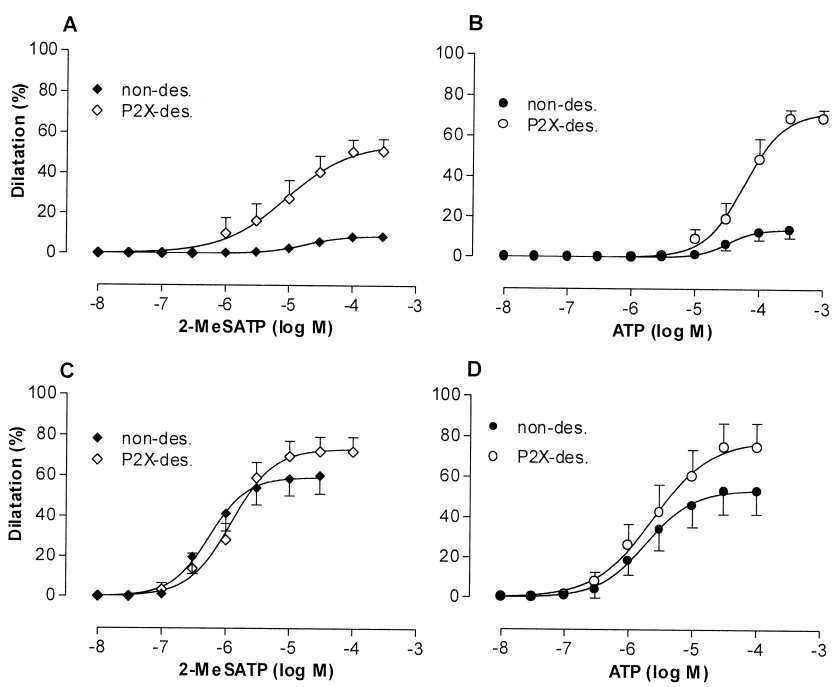

Fig. 3. Differential effects of P2X receptor desensitisation on EDHF- and NO-mediated dilatation. Concentration dependent dilatations to 2-MeSATP and ATP in the isolated mesenteric artery in the presence of indomethacin and L-NOARG (EDHF-dilatation; A and B) and indomethacin, charybdotoxin and apamin (NO-dilatation; C and D). Dilatations were examined without (solid symbols) and with (open symbols) prior P2X receptor desensitisation (10^{-5} M αβ-MeATP) (data also shown in Fig. 1A and B, respectively). Responses are expressed as percentage of an initial contraction induced by 10^{-6} M noradrenaline and are shown as mean ± S.E.M. of eight experiments.

traction. After a period of 8 min, the tension was back to baseline and if αβ-MeATP was added a second time, no contraction was observed, indicating desensitised P2X receptors. This did not affect the magnitude of noradrenaline precontraction (6.3 ± 0.4 mN without, and 6.6 ± 0.5 mN with prior P2X receptor desensitisation).

The *EDHF-mediated dilatation* was studied in the presence of L-NOARG and indomethacin, and was most potently stimulated by ADPβS ($pEC_{50} = 6.6 \pm 0.1$), while 2-MeSATP and ATP were almost without effects (Table 1 and Fig. 1A). UTP also elicited potent dilatations ($pEC_{50} = 5.1 \pm 0.1$), while UDP had only minor effects ($E_{max} = 29 \pm 4\%$). When pretreated with hexokinase, UDP was almost inactive ($E_{max} = 8 \pm 2\%$, $P < 0.05$) (Table 1). After P2X receptor desensitisation, ATP and 2-MeSATP demonstrated significantly more efficacious EDHF-mediated dilatory responses ($E_{max} = 14 \pm 4\%$ and $9 \pm 2\%$ before, and $69 \pm 5\%$ and $51 \pm 6\%$ after P2X receptor desensitisation, respectively, $P < 0.05$), with no change in potency, while the ADPβS and UTP dilatations were not affected (Table 1 and Figs. 1B and 3A,B). The difference in ATP and 2-MeSATP dilatation could not be reproduced after a short noradrenaline contraction.

The *NO-mediated dilatation* was studied in the presence of charybdotoxin, apamin and indomethacin. ADPβS, 2-MeSATP, ATP and UTP elicited potent and efficacious dilatations with a pEC_{50} of approximately 6, while UDP had only minor effects ($E_{max} = 28 \pm 5\%$ in the absence, and $20 \pm 5\%$ in the presence of hexokinase) (Table 1 and Fig. 2). The efficacy of the NO-mediated dilatory responses to ATP and 2-MeSATP was only slightly attenuated by P2X receptor desensitisation ($E_{max} = 53 \pm 1\%$ and $75 \pm 12\%$ before, and $60 \pm 9\%$ and $72 \pm 7\%$ after P2X receptor desensitisation, respectively, not significant), while the response to ADPβS and UTP were unchanged (Table 1 and Fig. 3C,D).

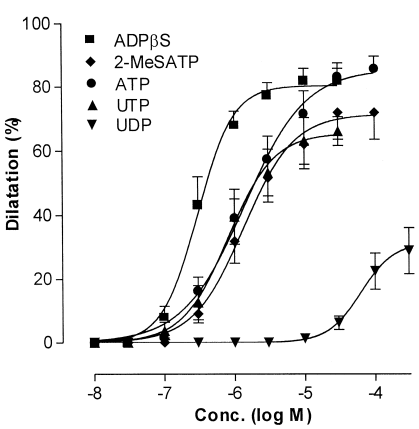

Fig. 4. *Total dilatations* were examined in the isolated mesenteric artery in the presence of indomethacin (10^{-5} M). After precontraction with 10^{-6} M noradrenaline, dilatory concentration–response curves for ADPβS, 2-MeSATP, ATP, UTP and UDP were constructed. Data are shown as mean ± S.E.M. of eight experiments.

The ''total dilatation'' was studied in the presence of indomethacin alone. Concentration–response curves that were similar to the NO-mediated dilatations could be observed for each of the different agonists. Thus, ADPβS, 2-MeSATP, ATP and UTP elicited potent and efficacious dilatations with a pEC_{50} of approximately 6, while UDP had only minor effects (Table 1 and Fig. 4).

In the presence of indomethacin, L-NOARG, charybdotoxin and apamin, the dilatory effects of ADPβS, 2-MeSATP, ATP, UTP or UDP were totally inhibited.

4. Discussion

The endothelium has emerged as an important regulator of vascular tone (Furchgott and Vanhoutte, 1989; Moncada et al., 1991), and stimulation of endothelial receptors by, e.g., acetylcholine release soluble mediators including NO, EDHF and prostaglandins that dilate vascular smooth muscle cells. The dilatory effects of purines and pyrimidines have so far mainly been characterised as an endothelium dependent effect mediated by NO or prostaglandins. We have recently shown that P2Y receptors induce EDHF release from the endothelium and thereby hyperpolarisation and dilatation of smooth muscle cells. These responses are antagonised by a combination of the K^+ channel inhibitors charybdotoxin and apamin (Malmsjö et al., 1998, 1999). Charybdotoxin and apamin selectively inhibits hyperpolarisation and dilatation mediated by EDHF (Corriu et al., 1996; Zygmunt and Högestätt, 1996; Zygmunt et al., 1998; White and Hiely, 1997; Chataigneau et al., 1998). The present study was designed to separately examine the EDHF- and NO-mediated dilatation in the rat isolated mesenteric artery.

Both endothelium denudation, as well as a combination of inhibitors of prostaglandins (indomethacin), EDHF (charybdotoxin and apamin) and NO (L-NOARG), abolished dilatation to each of the different agonists used; ADPβS, 2-MeSATP, ATP, UTP and UDP. We have previously shown that indomethacin only slightly attenuated the ATP dilatation (less than 10%) in the presence of L-NAME (Malmsjö et al., 1998). Thus, the dilatory responses in this preparation are mediated by the endothelium-dependent mediators EDHF and NO, with only a minor contribution of prostaglandins. The EDHF-mediated dilatation was studied in the presence of indomethacin and L-NOARG, in order to eliminate prostaglandin and NO-mediated effects. The NO-mediated dilatation was studied in the presence of indomethacin, charybdotoxin and apamin, in order to eliminate prostaglandin and EDHF-mediated effects.

4.1. Effects of P2X receptor desensitisation

The EDHF-mediated dilatation was most potently induced by ADPβS, while 2-MeSATP and ATP were inac-

tive. This pharmacological profile is not consistent with any of the so far cloned P2Y receptors. However, after desensitisation of P2X receptors with αβ-MeATP (see methods), 2-MeSATP and ATP were significantly more efficacious. The NO-mediated dilatory responses to ATP and 2-MeSATP were only slightly increased by P2X receptor desensitisation (Fig. 3). These results suggest that the EDHF-mediated dilatation is counteracted by simultaneous activation of P2X receptors on smooth muscle cells, while the NO-mediated dilatation is mainly unaffected. The difference in responses may be explained by the hyperpolarising effect of EDHF, which may be counterbalanced by a depolarising effect by the simultaneous activation of P2X receptors on smooth muscle cells. Since the NO-mediated dilatation is accompanied by a minor hyperpolarisation (Murphy and Brayden, 1995), the slight difference in NO-mediated dilatation after P2X receptor desensitisation might be caused by a similar mechanism. Another possible explanation of the counter-regulatory effect of P2X receptors is purely mechanistic; contraction elicited by P2X receptors counteracts the dilatation mediated by EDHF and NO. This is in accordance with the different potencies of the responses. ATP stimulates NO-mediated dilatation at a pEC_{50} of 5.7 and EDHF-mediated dilatation at 4.4. Contraction via P2X receptor is stimulated by ATP at a pEC_{50} of 4.1 in the same preparation (not published data), which might explain why EDHF is more affected than the NO dilatation. However, when measuring the membrane-potential before and after P2X receptor desensitisation in the isolated mesenteric artery pretreated with indomethacin and L-NOARG, the hyperpolarisation produced by ATP and 2-MeSATP was abolished (Malmsjö et al., 1999), making a membrane potential modulation more probable (Fig. 5).

To evaluate a possible non-specific effect of the αβ-MeATP induced contraction on the subsequent EDHF-mediated dilatations, αβ-MeATP was substituted for a short noradrenaline contraction (see methods). This did not affect the ATP or 2-MeSATP responses, suggesting that the effect of P2X receptor desensitisation is not due to an unspecific contractile effect of αβ-MeATP.

Dilatations induced by ADPβS and UTP were unaffected by P2X receptor desensitisation, both for the EDHF and NO-mediated responses, probably because these compounds are more specific and do not stimulate P2X receptors. $P2Y_1$ receptors are endothelial, dilatory receptors and are selectively stimulated by ADPβS (Ralevic and Burnstock, 1998). On the other hand, UTP stimulates $P2Y_2$ and $P2Y_4$ receptors that are located both on the endothelium and on the smooth muscle cells. Since UTP only induces contraction in high concentrations (approximately 1 mM, not published data), the contractile effect of this compound may not interact with its dilatory effects.

ATP from local sources such as platelets, sympathetic nerves and endothelial cells may activate P2X receptors on vascular smooth muscle cells. Here we have provided the first evidence that activation of P2X receptors efficiently can reduce the dilatory effect of EDHF. Consequently, we suggest that P2X receptors may act to counter-balance EDHF by reducing its hyperpolarising effects. ATP may in fact be released in parallel with EDHF from endothelial cells during hypoxia, shear stress or dilatory substances such as substance P, acetylcholine and bradykinin. Thus, the relationship between the amounts of ATP released and EDHF is of importance for the vascular dilatory response. Differential distribution and expression of P2X receptors may, e.g., in congestive heart failure, influence the EDHF-mediated vascular dilatation (Malmsjö et al., 1999a,b).

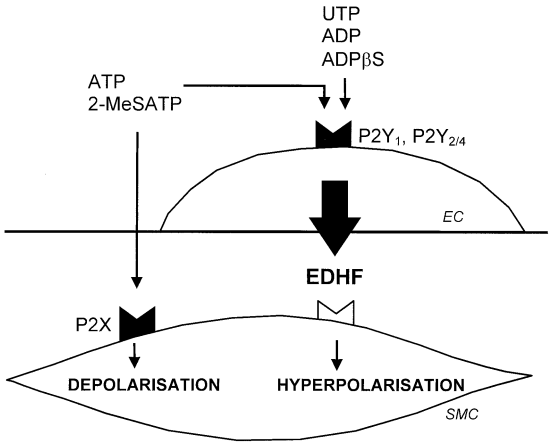

Fig. 5. ATP, 2-MeSATP, ADP, ADPβS and UTP activate P2Y-receptors located on endothelial cells (EC), and stimulate release of EDHF that hyperpolarises and relaxes vascular smooth muscle cells (SMC). In addition, ATP and 2-MeSATP stimulate P2X receptors on smooth muscle cells that induce depolarisation and thereby counteract the EDHF effect. After P2X receptor desensitisation with αβ-MeATP the EDHF mediated dilatation is revealed.

4.2. P2Y receptor mediated dilatory responses

ADPβS was thus the most potent agonist at inducing EDHF-mediated dilatation. After P2X receptor desensitisation, 2-MeSATP and ATP were both full agonists, although less potent, which is consistent with agonist potency of the cloned $P2Y_1$ receptor (Fig. 1B) (Léon et al., 1997). Potent NO-mediated effects could also be observed by ADPβS, 2-MeSATP and ATP, suggesting stimulation of both EDHF and NO release via $P2Y_1$ receptors (Fig. 2). ATP (after P2X receptor desensitisation) and UTP induced both EDHF and NO-mediated dilatations, suggesting involvement of $P2Y_2$ or $P2Y_4$ receptors (Figs. 1B and 2). UDP only stimulated dilatations with low efficacy. To increase purity of UDP, hexokinase and glucose were used to convert any contaminating UTP to UDP. After this treatment the EDHF-mediated dilatation was negligible, and the NO response was significantly decreased indicating very minor effects of $P2Y_6$ receptors. The effect was probably not due to an unspecific effect of hexokinase or

glucose, as they neither possessed any contractile or dilatory properties, nor did they modulate the magnitude of the noradrenaline precontraction.

4.3. The dual regulation of vascular dilatation by EDHF and NO

The pharmacological profile of the P2Y receptor mediated responses was comparable for the total dilatation of the vessel (studied in the presence of indomethacin alone) (Fig. 4), and the NO- and EDHF-mediated dilatation after P2X receptor desensitisation. The fractions of dilatation produced by NO and EDHF for each of the different receptors were similar, which is in agreement with that the same second messenger systems are activated by the different P2Y receptor subtypes and stimulate the production and release of both NO and EDHF.

The dilatory responses were mediated most potently by NO, which resembled the total dilatation of the vessel, and in slightly higher agonist concentrations by EDHF. These findings are consistent with the view that EDHF may provide a secondary system to NO in the mesenteric artery. However, the physiological importance of EDHF may increase in smaller resistance blood vessels (Garland et al., 1995).

The additive dilatory response of NO and EDHF for respective agonist exceeds the total dilatory response by far. Rather than functioning in an additive way, NO or EDHF might separately provide systems that are capable of fully dilating the blood vessels independently. This may be a reserve mechanism, where one factor is backup for the other if affected by pathological conditions like endothelium dysfunction in congestive heart failure, hypercholesterolemia, hypertension or diabetes (Vargas et al., 1996; Bauersachs et al., 1996; Brandes et al., 1997; Makino and Kamata, 1998; Malmsjö et al., 1999b).

4.4. Summary

Both NO- and EDHF-mediated dilatations can be induced by activation of $P2Y_1$ and $P2Y_2/P2Y_4$ receptors, with a minor role for $P2Y_6$ receptors. The EDHF-mediated dilatation was counteracted by the depolarising effects of P2X receptor activation on smooth muscle cells upon simultaneous stimulation by 2-MeSATP or ATP (Fig. 5). These results suggest a counter-regulatory role for P2X receptors to EDHF-mediated dilatations.

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