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Relaxant effect of 2-methyl-thio-adenosine diphosphate on rat thoracic aorta: effect of clopidogrel

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Abstract

The main aim of this study was to determine the functional effect of 2-methyl-thio-adenosine diphosphate (2MeS-ADP) on vascular purinoceptors, in comparison with that of a characterised agonist of the P2Y1 receptor, 2-methyl-thio-adenosine triphosphate (2MeS-ATP), and of the P2Y2 receptor, uridine triphosphate (UTP). On phenylephrine-precontracted rat aortic rings, mounted isometrically in organ baths, we found that 2MeS-ADP (10^{-9} to 10^{-6} M) induced concentration-dependent relaxation of rings with a functional endothelium. Mechanical removal of the endothelium abolished the relaxant effect of 2MeS-ADP. The 2MeS-ADP-induced relaxation of phenylephrine-precontracted rings was inhibited by *N*ω-nitro-L-arginine methyl ester (L-NAME) (100 μM) but not by indomethacin (100 μM) or aspirin (1 mM), indicating that the 2MeS-ADP-induced relaxation was nitric oxide (NO) synthase-mediated but not cyclooxygenase-dependent. Repeated stimulation with 2MeS-ADP resulted in desensitisation of the receptor. Under these conditions, the relaxant effect of 2MeS-ATP was abolished. On the contrary, UTP-induced relaxation was not affected, showing that 2MeS-ADP and 2MeS-ATP but not UTP shared the same receptor. Suramin (100 μM), a non-specific P2 inhibitor, abolished the effect of 2MeS-ADP, 2MeS-ATP and UTP. In contrast, pyridoxal-phosphate-6-azophenyl-2′-4′-disulphonic acid (PPADS) and adenosine-3′-phosphate-5′-phosphosulphate (A3P5PS) abolished only the vasodilator responses to 2MeS-ADP and 2MeS-ATP and did not affect the relaxant effect of UTP, showing that 2MeS-ADP acted through the P2Y1 receptor. Clopidogrel, a potent platelet ADP receptor antagonist, at a dose that strongly inhibited ADP-induced platelet aggregation ex vivo, did not modify the relaxant responses to 2MeS-ADP or 2MeS-ATP. In conclusion, these results showed that 2MeS-ADP induces endothelium-dependent, NO-mediated relaxation of rat aortic rings. This effect, resistant to clopidogrel treatment, occurred through activation of the P2Y1 receptor.

Keywords: ADP; ATP; UTP; Purinoceptor; Thoracic aorta; Clopidogrel

1. Introduction

ATP, 2MeS-ATP, UTP, ADP and ADPβS have been shown to be vasorelaxant agents in various experimental models (for review, see Ralevic and Burnstock, 1991). They can cause both vasoconstriction, mainly by activation of purinoreceptors located on smooth muscle cells, and vasodilatation, mainly by activation of endothelial purinoreceptors. Several purinoreceptors have been described in many cells. This receptor family, named P2, is taken to be composed of 14 different cloned receptors classified into two subfamilies: P2X (7 members) are pore-forming protein sequences and P2Y (7 members) are G protein-coupled receptors (Fredholm et al., 1997). Platelet ADP receptors (named P2T) which have not yet been cloned, have been classified with the P2Y subfamily. In contrast to the other P2 receptors, diphosphate derivatives of adenosine act as agonists whereas the corresponding triphosphates behave as antagonists (Macfarlane et al., 1983). P2Y1 and P2Y2 (previously named P2U) present on endothelial cell or smooth muscle cells, have been described by several authors as mediators of vasorelaxation (Kennedy et al., 1985; Motte et al., 1993; Wilkinson et al., 1994; Garcia-Velasco et al., 1995; Hansmann et al., 1997). The most potent agonists of these two receptors are 2-methyl-thio-adenosine triphosphate (2MeS-ATP) for P2Y1, and uridine triphosphate (UTP) for P2Y2 (Dainty et al., 1991; Schachter et al., 1996; Hansmann et al., 1997).

We now first characterised the effects of an analogue of adenosine diphosphate, 2-methyl-thio-adenosine diphosphate (2MeS-ADP) on endothelial cells. 2MeS-ADP has already been described as the most potent agonist at the ADP receptor on platelets (currently named P2T) (Mac-

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farlane et al., 1983). It has also been demonstrated to be a potent agonist of the cloned human or turkey P2Y1 receptor (Schachter et al., 1996) but, until now, a vasoactive effect of 2MeS-ADP on endothelial cells has not been shown. The experiments were done with a large isolated artery (rat thoracic aorta) in which P2Y1 and P2Y2 receptors have been described (Dainty et al., 1991; Saïag et al., 1996; Hansmann et al., 1997).

In the second part, we studied the effect of clopidogrel, a thienopyridine which has shown strong anti-aggregant and anti-thrombotic activities in many studies (for review, see Herbert et al., 1993). Recently, in a large clinical trial, clopidogrel has been demonstrated to be efficacious to prevent thrombotic events in atherosclerotic patients (Caprie Steering Committee, 1996). Clopidogrel treatment has been found to specifically antagonise the binding of ADP to its platelet receptors and to consequently inhibit platelet aggregation (Mills et al., 1992; Savi et al., 1994a,b). Although the exact nature of the purinoreceptor responsible for ADP-induced platelet aggregation (the target of clopidogrel on platelets) is still unknown, the presence of the P2Y1 receptor at the platelet surface has been demonstrated recently but its role in platelet aggregation is still not completely understood (Léon et al., 1997; Hechler et al., 1998b; Daniel et al., 1998; Savi et al., 1998). Indeed, it is now clear that ADP-induced aggregation cannot be attributed to activation of the P2Y1 receptor alone but should be attributed to the simultaneous activation of a high-affinity (P2Y1) and a low-affinity (P2T, the target of clopidogrel) receptor of ADP (Savi et al., 1998). In order to determine if such ADP receptor sites exist on endothelial cells, we examined the vasoactive effect of 2MeS-ADP and determined the effect of clopidogrel treatment on 2MeS-ADP-induced vasorelaxation.

2. Materials and methods

2.1. Isolated rat thoracic aorta

Male Sprague–Dawley rats (350–450 g, Iffa-Credo, L'Arbesle, France) were killed by cervical dislocation after pentobarbital anaesthesia (50 mg/kg, i.p.) and the thoracic aorta was rapidly removed and placed in an oxygenated (O_2 95%, CO_2 5%) Krebs solution (composition mmol/l: NaCl 112, KCl 5, $NaHCO_3$ 25, KH_2PO_4 1, $MgSO_4$ 1.2, $CaCl_2$ 1.25, glucose 11.5). This protocol was approved by the Animal Care and Use Committee of Sanofi Recherche. The isolated aorta was then cut into rings (3–4 mm length) which were mounted between two stainless-steel hooks in an automated isolated organ system (Emka Technologies, Paris, France). Rings were set up under 2 g of tension and equilibrated for 1 h in an oxygenated Krebs solution which was changed every 15 min. A first contraction in response to phenylephrine (1 μM) was then recorded, followed by a relaxation with acetylcholine (1 μM) which was more than 70% in endothelium-intact rings and served as a control of endothelial integrity. The concentration–response relationships of 2MeS-ADP, 2MeS-ATP, ADP, ATP or UTP were investigated as follows: after the test with acetylcholine, the rings were washed 3 times for a 40 min period. After return to baseline, the preparation was again precontracted with phenylephrine (1 μM) and, when the plateau of the phenylephrine-induced contraction was stable (after 5 min), increasing concentrations (3.10^{-9} to 10^{-5} M) of nucleotides were added cumulatively. Finally, the preparation was washed for 30 min and the functional state of the endothelium was again verified by reapplying acetylcholine (1 μM). In some cases, the endothelium was removed by gentle rubbing of the intimal surface. In order to determine the effect of inhibitors, aortic rings were contracted with phenylephrine (1 μM) and a first relaxation in response to 2MeS-ADP, 2MeS-ATP and UTP was recorded. The rings were then rapidly washed and incubated for three 10 min periods separated by washes before a second contraction to phenylephrine was induced. Antagonists were injected just after the last wash.

2.2. Platelet aggregation experiments

Platelet-rich plasma was prepared from 5 ml of blood taken and mixed with 3.8% sodium citrate (9/1, v/v) as described previously (Savi et al., 1994a). Aggregation was measured at 37°C, using a turbidimetric method in a dual-channel Chrono-Log aggregometer. The platelet rich plasma was equilibrated for 1 min under constant stirring (900 rpm) and aggregation was induced by addition of the agonists (Savi et al., 1992).

2.3. Conversion of diphosphates nucleotides by ATP generating system

Treatment of commercial preparations of 2MeS-ATP and 2MeS-ADP with the ATP-generating system creatine phosphate/creatine phosphokinase was performed according to the work of Hechler et al. (1998b). Briefly, 0.1 mM nucleotides was incubated at room temperature with 20 U/ml creatine phosphokinase and 10 mM creatine phosphate in a Krebs buffer for 90 min. The mixtures were then used as inducers in rat aorta relaxation and rat platelet aggregation experiments.

2.4. Ex vivo experiments

In order to determine the influence of clopidogrel on the 2MeS-ADP- or 2MeS-ATP-induced relaxations, clopidogrel and its inactive enantiomer, SR25989C, were given to rats orally, 25 mg/kg, for 2 days. The last administration was performed 2 h before blood and aorta sampling. The anti-aggregating effect of clopidogrel was determined by measuring the ADP (2.5 μM)-induced aggregation of

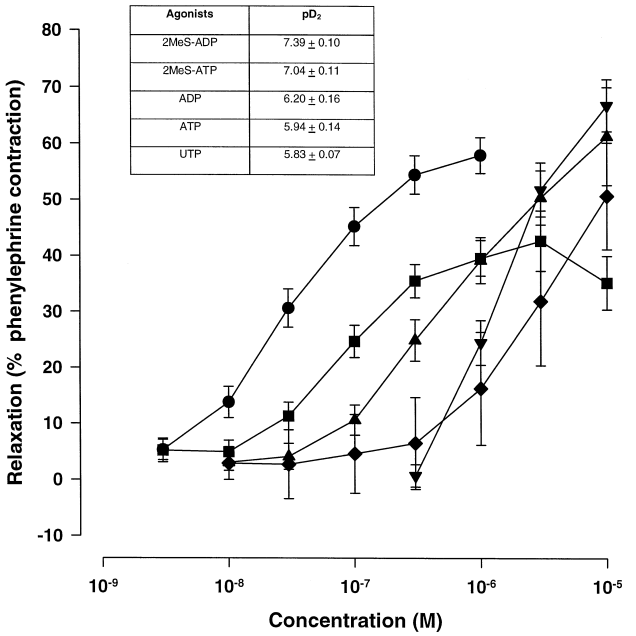

Fig. 1. Relaxation of the rat thoracic aorta by 2-methyl-thio-adenosine diphosphate (2MeS-ADP) (●), 2-methyl-thio-adenosine triphosphate (2MeS-ATP) (■), adenosine diphosphate (ADP) (▲), uridine triphosphate (UTP) (▼) and adenosine triphosphate (ATP) (◆) expressed as percent reversal of phenylephrine (1 μM)-induced contraction. The nucleotides were added cumulatively 5 min after phenylephrine. Each point is the mean ± S.E.M. for 3–9 rats.

platelet rich plasma as described above. The rings were prepared and the effect of 2MeS-ADP and 2MeS-ATP was evaluated with the protocol described above.

2.5. Analysis of the results

The relaxant effect of the various agonists was expressed as a percentage of phenylephrine-induced contraction. The individual dose–response curves were fitted to a logistic equation in order to calculate pD_2 (the negative log of these EC_{50} values). For statistical analysis of the results, the pD_2 values were compared using an analysis of variance (ANOVA) followed by the Dunnet's multiple comparison test. Data are presented as means ± S.E.M. from *n* rats.

2.6. Drugs

2MeS-ADP (sodium salt) and 2MeS-ATP (tetrasodium salt) and pyridoxal-phosphate-6-azophenyl-2′-4′-disulphonic acid 4-sodium (PPADS) were obtained from Research Biochemicals (Natick, MA, USA). UTP (sodium salt), ATP (disodium salt) and ADP (sodium salt) were obtained from Boehringer Mannheim (Penzberg, Germany). Acetylcholine chloride, phenylephrine, indomethacin (in stock solution in ethanol 10^{-2} M), *N*ω-nitro-L-arginine methyl ester (L-NAME), *N*ω-nitro-D-arginine methyl ester (D-NAME), creatine phosphate, creatine phosphokinase and adenosine-3′-phosphate-5′-phosphosulphate (A3P5PS) were obtained from Sigma (L'isle d'Arbeau, France). Suramin was purchased from Calbiochem (La Jolla, CA, USA). Aspirin (Aspegic®) was from Synthelabo (Le Plessis-Robinson, France). Clopidogrel (Plavix®) and SR25989C were obtained from Sanofi Recherche (Toulouse, France).

3. Results

3.1. Relaxant effect of 2MeS-ADP

2MeS-ADP, at nanomolar concentrations, induced a significant relaxation of phenylephrine (1 μM)-precontracted rings of rat thoracic aorta (Fig. 1). 2MeS-ADP-in-

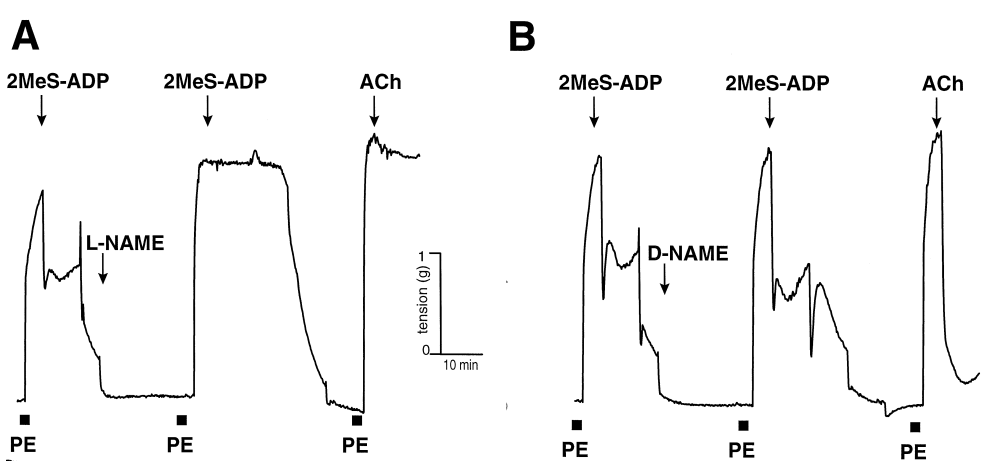

Fig. 2. Effect of *N*ω-nitro-L-arginine methyl ester (L-NAME) or *N*ω-nitro-D-arginine methyl ester (D-NAME) on the relaxant effect of acetylcholine and 2MeS-ADP. A first contraction in response to phenylephrine (1 μM) and relaxation with 2MeS-ADP (1 μM) were followed by several washes. L-NAME (100 μM) (A) or D-NAME (100 μM) (B) was preincubated for 30 min before a second contraction with phenylephrine and a relaxation to 2MeS-ADP were recorded. After a second period of washes, a third contraction with phenylephrine followed by a relaxation with acetylcholine was recorded. Acetylcholine or 2MeS-ADP was added 5 min after phenylephrine. The result shown is from a typical experiment, representative of three others.

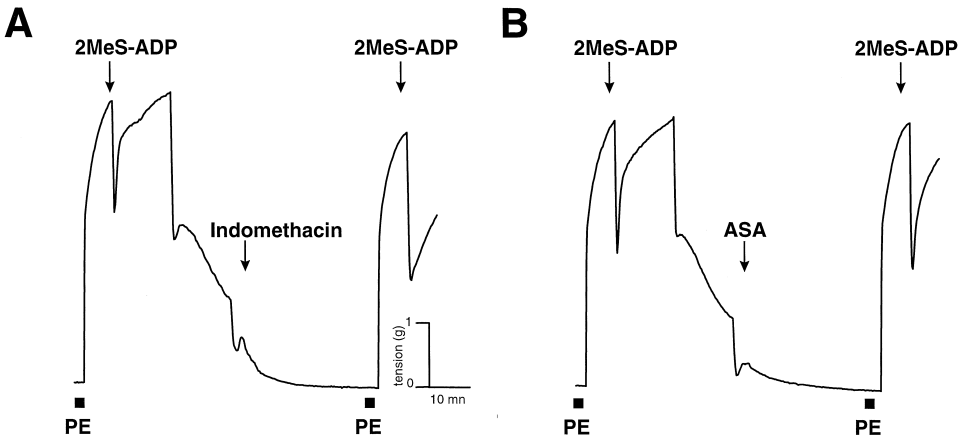

Fig. 3. Effect of indomethacin or aspirin on the relaxant effect of 2MeS-ADP. A first contraction induced by phenylephrine (1 μM) and relaxation by 2MeS-ADP (1 μM) were followed by several washes. Indomethacin (100 μM) (A) or aspirin (ASA, 1 mM) (B) was preincubated for 30 min before a second contraction with phenylephrine and a relaxation with 2MeS-ADP were recorded. The result shown is from a typical experiment, representative of three others.

duced relaxation was concentration-dependent with a pD_2 of 7.39 ± 0.1 ($n = 9$) (Fig. 1). The maximum relaxation (58%) was obtained for 1 μM and a tachyphylactic effect was observed with higher concentrations (not shown). After the mechanical removal of the endothelium, the acetylcholine (1 μM)- and 2MeS-ADP-induced relaxation of the rings was totally suppressed (not shown). As shown in Fig. 2A in phenylephrine-precontracted rings with an intact endothelium, L-NAME (100 μM) totally inhibited 2MeS-ADP-induced relaxation. A similar effect also occurred with regard to acetylcholine (1 μM)-induced relaxation (Fig. 2A). D-NAME at the same concentration had no effect (Fig. 2B). Preincubation of the rings with indomethacin (100 μM) or aspirin (1 mM) did not influence the 2MeS-ADP-induced relaxation (Fig. 3). At these concentrations, inhibition of cyclooxygenase by indomethacin or aspirin was confirmed by the ability of these compounds to inhibit contractions induced by arachidonic acid (100 μM) (data not shown).

2MeS-ADP, 2MeS-ATP, ADP, UTP and ATP relaxed the rat thoracic aorta with an agonist potency order of 2MeS-ADP > 2MeS-ATP ≫ ADP > ATP ≃ UTP (Fig. 1). As shown in Fig. 1, 2MeS-ADP was more potent than 2MeS-ATP, the difference between the two pD_2 values being statistically significant (Dunnet's test; $P = 0.04$).

Table 1
Potencies of 2MeS-ADP and 2MeS-ATP to induce relaxation of phenylephrine-precontracted rat aortic rings or the aggregation of platelets after treatment with creatine phosphate/creatine phosphokinase (CP/CPK)

	Relaxation (%)		Aggregation (%)	
	−CP/CPK	+CP/CPK	−CP/CPK	+CP/CPK
2MeS-ADP (1 μM)	39 ± 3	34 ± 3	34	7
2MeS-ATP (1 μM)	30 ± 9	42 ± 14	35	7

Values are means ± S.E.M.; $n = 2$–4.

Since it has been demonstrated that commercial preparations of 2MeS-ATP could be contaminated with 2MeS-ADP (Léon et al., 1997; Hechler et al., 1998a), we verified the effects of 2MeS-ATP on rat aorta and rat platelets after treatment with the creatine phosphate/creatine phosphokinase ATP regenerating system. Under the conditions described by these authors, incubation of 2MeS-ADP with

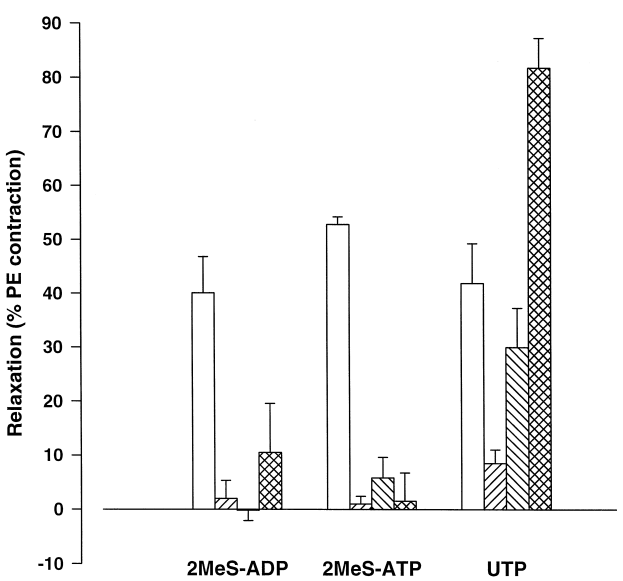

Fig. 4. Effect of P2Y receptor antagonists on the relaxant effect of 2MeS-ADP, 2MeS-ATP and UTP in rat aortic rings. A first contraction in response to phenylephrine (1 μM) and a relaxation in response to 2MeS-ADP (1 μM) or 2MeS-ATP (1 μM) or UTP (3 μM) were followed by several washes. Suramin (100 μM; rectangle with right-to-left diagonal lines), pyridoxal-phosphate-6-azophenyl-2′-4′-disulphonic acid 4-sodium (PPADS) (100 μM; rectangle with left-to-right diagonal lines) or adenosine-3′-phosphate-5′-phosphosulphate (A3P5PS) (250 μM; rectangle with intersecting diagonal lines) was preincubated for 5 min before a second contraction with phenylephrine and relaxation with the same nucleotides were recorded (control without the antagonists; hollow rectangle).

creatine phosphate/creatine phosphokinase abolished the aggregating effect of this inducer. In the same way, the aggregating effect observed with a commercial preparation of 2MeS-ATP was eliminated, indicating that traces of 2MeS-ADP were responsible for this activity. However, rat aorta relaxation was still observed after creatine phosphate/creatine phosphokinase treatment of both nucleotides, indicating that 2MeS-ATP acts as an agonist on the rat thoracic aorta (Table 1).

Serial additions of 2MeS-ADP (1 μM) abolished the relaxant effect of 2MeS-ADP, showing desensitisation of the receptor. After total disappearance of the 2MeS-ADP-induced relaxation, the subsequent injection of 2MeS-ATP (1 μM) did not produce any relaxant effect, whereas UTP was still able to induce relaxation of the rings (not shown).

To further characterise the receptor by which 2MeS-ADP relaxed the aortic ring, we determined the effect of suramin and PPADS, two non-selective P2 receptor antagonists and of A3P5PS, a selective P2Y1 receptor antagonist. As shown in Fig. 4, suramin (100 μM) totally abolished the relaxant effect of 2MeS-ADP, 2MeS-ATP and UTP. PPADS (100 μM) strongly inhibited 2MeS-ADP and 2MeS-ATP-induced relaxations but only slightly inhibited UTP-induced relaxation. A3P5PS (250 μM) strongly inhibited 2MeS-ADP and 2MeS-ATP-induced relaxation but did not inhibit UTP-induced relaxation. Suramin, PPADS and A3P5PS at the concentrations used had no influence on phenylephrine-induced contractions (data not shown).

3.2. Treatment with clopidogrel

Clopidogrel and its inactive enantiomer (SR25989C) were given orally (25 mg/kg) to rats 24 and 2 h before killing. Under these conditions, clopidogrel inhibited the ADP(2.5 μM)-induced platelet aggregation by 76.6 ± 4.2% ($P < 0.05$, $n = 14$). SR25989C had no effect on ADP-induced platelet aggregation (13.4 ± 5.1% inhibition, ns, $n = 5$), in accordance with previously published results (Herbert et al., 1993). The relaxant effects of 2MeS-ADP and 2MeS-ATP were studied on phenylephrine-precontracted rings of clopidogrel- and SR25989C-treated rats and were compared with that in the placebo-treated group. As shown in Table 2, neither clopidogrel nor SR25989C modified the relaxant effect of 2MeS-ADP or 2MeS-ATP and no significant differences between these pD_2 were found (ANOVA; ns).

Table 2
Potencies of 2MeS-ADP and 2MeS-ATP to induce the relaxation of phenylephrine-precontracted rat aortic rings, after treatment with clopidogrel or SR25989C

pD_2			
Agonist	Vehicle	Clopidogrel	SR25989C
2MeS-ADP	7.39 ± 0.10	7.26 ± 0.05	7.32 ± 0.06
2MeS-ATP	7.04 ± 0.11	7.04 ± 0.20	6.86 ± 0.07

Values are mean pD_2 ± S.E.M.; $n = 4–14$ rats.

4. Discussion

We now showed that exposure to 2MeS-ADP led to the relaxation of phenylephrine-precontracted rings of rat thoracic aorta. This effect was dependent on the presence of the endothelium and was abolished after mechanical removal of the endothelium. 2MeS-ADP induced the relaxation of phenylephrine-contracted rat aortic rings through a nitric oxide synthase-dependent pathway, as shown by the inhibitory effect of L-NAME, a selective inhibitor of NO synthase. In contrast, cyclooxygenase block by either indomethacin or aspirin did not alter the effect of 2MeS-ADP, indicating that 2MeS-ADP-induced vasorelaxation was not dependent on prostaglandins and prostacyclin release. This result is consistent with the conclusions of Ralevic and Burnstock (1996) for isolated rat mesenteric arteries or those of Eltze and Ullrich (1996) who showed that stimulation of the P2Y receptor involves NO synthesis, but not the release of cyclooxygenase-derived mediators. Conversely, Saïag et al. (1996) and Wilkinson et al. (1994) found that P2Y1-mediated vasorelaxing effects were mediated by NO synthase and cyclooxygenase activation, in rat pancreatic vascular bed and bovine aortic collateral artery, respectively. One explanation for the differing conclusion about cyclooxygenase involvement could be differences between species and between vascular preparations.

In order to characterise the receptors involved in the vasoactivity of 2MeS-ADP, desensitisation experiments were performed. They showed that 2MeS-ADP did not desensitise the response to UTP (via the P2Y2 receptor), but shared the same receptor with 2MeS-ATP. This receptor has been defined as the P2Y1 receptor (Fredholm et al., 1994, 1997; Schachter et al., 1996), and its presence in endothelial cells has been demonstrated (Garcia-Velasco et al., 1995; Hansmann et al., 1997). The P2Y1 receptor has been defined as mainly activated by 2MeS-ATP but these results suggest that 2MeS-ADP is also an agonist of the rat endothelial P2Y1 receptor, which is consistent with the observation of Schachter et al. (1996) who found that 2MeS-ADP was a potent agonist of the cloned human and turkey P2Y1 receptors (Schachter et al., 1996). These findings were confirmed when we determined the effect of several P2 receptor antagonists. The paucity of selective antagonists of P2Y receptors impedes the characterisation of these receptors on various organs. The most commonly used antagonists are suramin and PPADS (Vials and Burnstock, 1994; Windscheif et al., 1995; Charlton et al., 1996; Ralevic and Burnstock, 1996; Hansmann et al., 1997). Suramin is a non-selective antagonist of P2Y receptors, as confirmed in our study where it inhibited 2MeS-ADP, 2MeS-ATP or UTP-induced relaxation. PPADS has been

described as a little more selective for the P2Y1 receptor (Charlton et al., 1996; Ralevic and Burnstock, 1996). We confirmed that PPADS was more selective than suramin since it inhibited 2MeS-ADP and 2MeS-ATP, but not UTP-induced relaxation. More recently, A3P5PS has been described as a competitive and selective antagonist of the cloned turkey P2Y1 receptor (Boyer et al., 1996). This was confirmed in the present study as A3P5PS was able to inhibit 2MeS-ADP and 2MeS-ATP-induced relaxation, but did not reduce the UTP-induced relaxation. Surprisingly, we observed a significant tendency to an increase of the UTP-induced relaxation with A3P5PS which has not been yet clarified.

This first report of the vasorelaxant effects of 2MeS-ADP on the receptor of 2MeS-ATP (P2Y1) confirms the finding that nucleotides diphosphates can stimulate the P2Y1 receptor with a potency comparable to that of the corresponding triphosphates. Recently, Hechler et al. (1998a) suggested that the 2MeS-ATP-induced Ca^{++} influx in P2Y1 receptor-transfected Jurkat cells was due to contaminating 2MeS-ADP in the reagent, 2MeS-ATP being an antagonist of this purinoceptor. Conversely, our results demonstrate that 2Me-ATP-induced relaxation of rat thoracic aorta cannot be attributed to 2MeS-ADP contamination, since treatment of the reagents with creatine phosphate/creatine phosphokinase did not modify the relaxant effect of 2MeS-ATP. Furthermore, relaxation was still observed after creatine phosphate/creatine phosphokinase treatment, whereas the same mixture failed to induce platelet aggregation.

Interestingly, 2MeS-ADP, shown here as the most potent vasorelaxant nucleotide, is also one of the most potent agonist of platelet activation (Macfarlane et al., 1983). This effect of 2MeS-ADP on platelet aggregation is mediated by the activation of both P2Y1 and P2T receptors (Savi et al., 1998). Clopidogrel administration inhibited the 2MeS-ADP-induced aggregation by affecting the [^{3}H]2MeS-ADP binding to the P2T receptor on platelets (Savi et al., 1994b). This effect was observed only after in vivo administration, clopidogrel being inactive in vitro. Since purinoceptors have been also found in the vascular wall, we tested if clopidogrel would affect the activity of ADP on one of these receptors. No pharmacological target other than platelets was found for clopidogrel. Therefore, treatment of rats with clopidogrel did not modify the vasorelaxant effects of 2MeS-ADP or 2MeS-ATP, indicating that the platelet 2MeS-ADP receptors antagonised by clopidogrel are different from that of the endothelial 2MeS-ADP receptors, P2Y1. This result confirms the conclusion of a recent study with rat platelets, showing that, after clopidogrel treatment, P2Y1 which is present on platelets was not affected (Savi et al., 1998). This result further shows that there is no vascular effect of clopidogrel, as had been suggested by Yang and Fareed (1997).

In conclusion, our study showed that 2MeS-ADP induces endothelium-dependent, NO-mediated relaxation of rat aortic rings. This effect, which is resistant to clopidogrel treatment, occurred through activation of the P2Y1 receptor.

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