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Short communication

ATP-induced, P_{2U} purinoceptor-mediated constriction of isolated, perfused mesenteric beds of the rat [1]

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Abstract

α,β-Methylene ATP (α, β-mATP), ATP and UTP dose dependently increased the perfusion pressure of rat mesenteric arteries with a potency order of α, β-mATP ≫ ATP > UTP. In the veins, while α, β-mATP did not affect the pressure, both ATP and UTP equi-potently increased it. The arterial ATP response was attenuated to some degree by suramin (100 μM), but markedly and to a similar extent by pyridoxal-phosphate-6-azophenyl-2′,4-disulphonic acid (PPADS 30 μM) and α, β-mATP (100 nmol). The venous response was not affected by PPADS or α, β-mATP, but was slightly attenuated by suramin. Thus, ATP seems to elicit arterial constriction predominantly by stimulating P_{2X}, but venous constriction by stimulating P_{2U} purinoceptors. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Mesenteric artery; Mesenteric vein; ATP; UTP; P_{2X} purinoceptor; P_{2U} purinoceptor

1. Introduction

In the perfused mesenteric arterial bed of the rat, ATP elicits vascular contraction through stimulation of P_{2X} purinoceptors on the smooth muscle cells, and relaxation through P_{2Y} purinoceptors on the endothelial cells (Ralevic and Burnstock, 1988). At P_{2X} purinoceptors, the potencies of agonists are ranked as α,β-methylene ATP (α,β-mATP) ≫ 2-methylthio ATP ≥ ATP, while at P_{2Y} purinoceptors they are ranked as 2 methylthio ATP ≫ ATP > α, β-mATP (Burnstock and Kennedy, 1985). On the other hand, UTP has almost no action at P_{2X} and P_{2Y} purinoceptors, but is an agonist at P_{2U} purinoceptors (O'Connor et al., 1991), and evokes both vasoconstriction and relaxation in the rat mesenteric bed (Ralevic and Burnstock, 1991). Besides, ATP is not only an agonist at P_{2X} and P_{2Y} purinoceptors but also acts as an agonist at P_{2U} purinoceptors (Ralevic and Burnstock, 1996; Hansmann et al., 1997; Malmsjö et al., 1998). In the present study we attempted to characterize the participation of P_{2U} purinoceptor-mediated events in ATP-induced vasoconstriction, using isolated, perfused mesenteric arteries and veins of rats. We hoped to clarify the specificity of the responses of different portions of the vessels.

2. Materials and methods

2.1. Isolated, separately perfused mesenteric arteries and veins

Male Wistar rats, 32 to 37 weeks old, weighing 487–630 g were used. The animals were anesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg/kg) and killed by exsanguination. The isolated, double perfused mesentery was prepared according to the method of Warner (1990). Briefly, polyethylene cannulae were inserted into the superior mesenteric artery and retrogradely into the portal vein. Modified Krebs–Henseleit solution, containing (in mM): NaCl 118, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25 and glucose, 11, warmed to 38°C and aerated with 95%O_2–5%CO_2 was sent through a circuit of vinyl tubes to the arterial cannula at a constant rate of 3–3.5 ml/min with a peristaltic pump (MP-3, Tokyo Rikakikai). The effluent from the whole mesenteric bed was drained for 5 min through the venous cannula to rinse blood away. The intestine was then re-

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moved from the mesentery by cutting the mesentery closely along the digestive tract. This procedure made it possible to perfuse the arterial and venous portions independently. Namely, the arteries were perfused in the regular direction from the cannula at the superior mesenteric artery, and the veins were perfused retrogradely, at the same rate as the arteries, by connecting the cannula at the portal vein to another perfusion circuit. After 45-min equilibration, the nucleotides were injected with a microsyringe into rubber tubes joined to the circuit at positions close to each cannula. The vascular responses were monitored as changes in perfusion pressure by a pressure transducer (P23XL, Spectramed) placed in the circuit between the outlet of the pump and the preparation for each arterial or venous portion.

2.2. Vascular responses to nucleotides and effects of antagonists

α, β-mATP (0.1–300 nmol), ATP and UTP (respective 0.1–3000 nmol) were injected and dose-response relationships were examined separately in the arteries and veins. In another series of experiments, responses to ATP and UTP (1000 and 3000 nmol, for each) were compared before and after the addition of reactive blue 2 (30 μM), suramin (100 μM) or pyridoxal-phosphate-6-azophenyl-2′,4-disulphonic acid (PPADS, 30 μM) to the physiological solution, or injecting α, β-mATP (100 nmol). The volume for injection was 10 μl to avoid changes in perfusion pressure due to the injection, and the dose of nucleotides, 3000 nmol/10 μl was the practical limit for the injection.

2.3. Chemicals

The following chemicals were used: ATP (Sigma-Aldrich Japan, Tokyo), α, β-mATP (Sigma-Aldrich Japan, Tokyo), PPADS (Funakoshi, Tokyo), reactive blue 2 (Funakoshi, Tokyo), suramin (Sigma-Aldrich Japan, Tokyo), UTP (Sigma-Aldrich Japan, Tokyo). All other chemicals were of the purest grade commercially available.

2.4. Statistics

The results are presented as means with standard errors. Statistical evaluation of the data before and after the treatment with antagonists was performed by Student's *t*-test for paired observations. Values were considered to be significantly different when P was less than 0.05.

3. Results

The nucleotides dose-dependently increased arterial perfusion pressure with a potency order of α, β-mATP ≫ ATP > UTP. In the veins, the dose-response curves for ATP and UTP were superimposed, and α, β-mATP failed to cause responses. The arterial responses to ATP and UTP were greater than dose-matched responses in the veins (Fig. 1). The effects of antagonists on the ATP and UTP responses are summarized in Fig. 2. Reactive blue 2 augmented the arterial response to ATP at 1000 and 3000 nmol to 173.8 ± 15.5% ($P < 0.001$, $N = 11$) and to 157.4 ± 10.4% ($P < 0.001$, $N = 11$) of the control, respectively. However, the arterial UTP responses were not changed by the agent. Suramin attenuated the arterial response to ATP at 1000 and 3000 nmol to 62.4 ± 9.6% ($P < 0.01$, $N = 7$) and to 76.6 ± 8.9% ($P < 0.05$, $N = 7$), and that to UTP at 1000 nmol and 3000 nmol to 60.5 ± 4.1% ($P < 0.001$, $N = 8$) and to 53.8 ± 6.1% ($P < 0.001$, $N = 8$), respectively. PPADS significantly inhibited the arterial response to ATP at 1000 and 3000 nmol to 22.8 ± 4.9% ($P < 0.001$, $N = 5$) and to 27.8 ± 5.1%, ($P < 0.001$, $N = 5$) of the control, respectively. On the other hand, PPADS potentiated the arterial response to UTP at 1000 and 3000 nmol to 198.8 ± 19.3% ($P < 0.01$, $N = 7$) and to 186.6 ± 10.6% ($P < 0.001$, $N = 7$), respectively. α, β-mATP attenuated the arterial response to ATP at 1000 and 3000 nmol to 17.0 ± 2.7% ($P < 0.001$, $N = 8$) and at to 20.5 ± 4.1% ($P < 0.001$, $N = 8$), respectively, but did not change the arterial UTP responses. The venous responses to both ATP and UTP were resistant to the antagonists, except suramin

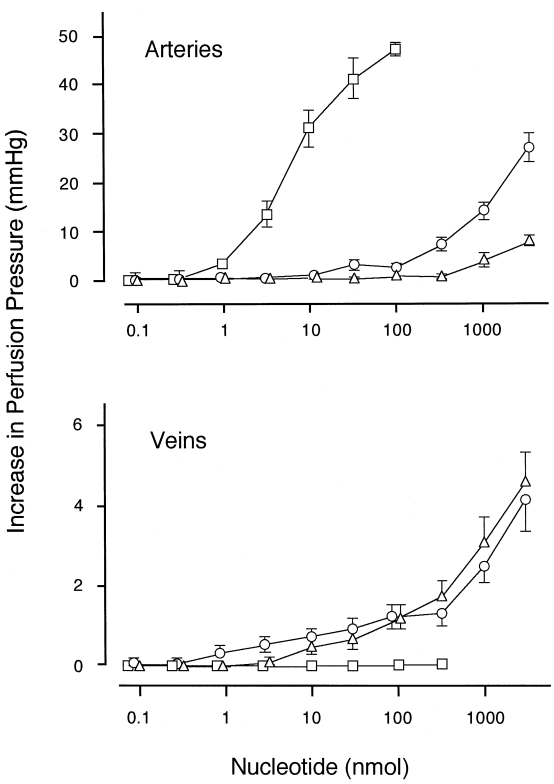

Fig. 1. Dose–response curves for nucleotides in isolated, perfused mesenteric arteries and veins of rats. Dose–response relationships for increases in perfusion pressure by ATP (circles), UTP (triangles) and α, β-mATP (squares) are given. Symbols with vertical bars represent means with S.E.M. for 8 experiments.

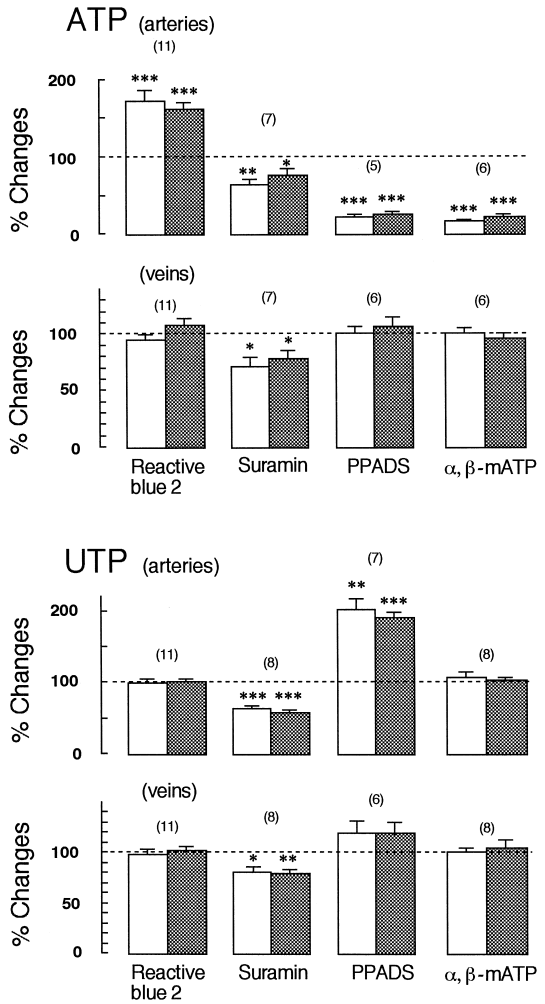

Fig. 2. Effects of P_2 purinoceptor antagonists on nucleotide-induced increases in perfusion pressure of isolated perfused mesenteric arteries and veins of rats. The responses to ATP or UTP (1000 and 3000 nmol) were compared before and after the addition of reactive blue 2 (30 μM), suramin (100 μM) or PPADS (30 μM) to the perfusion solution, or before and after the repeated injection of α, β-mATP (100 nmol). Open columns (responses to the nucleotides at 1000 nmol) and shaded columns (responses to the nucleotides at 3000 nmol) with vertical bars are percent means of the control responses with S.E.M. Numbers of experiments are in parentheses. $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$, significantly different from the control responses (100%).

which attenuated the response to ATP at 1000 and 3000 nmol to 68.3 ± 11.7% ($P < 0.05$, $N = 7$) and to 78.4 ± 8.5% ($P < 0.05$, $N = 7$), and that to UTP at 1000 and 3000 nmol to 79.9 ± 8.0% ($P < 0.05$, $N = 8$) and to 78.9 ± 4.5% ($P < 0.01$, $N = 8$), respectively.

4. Discussion

The potency rank of the nucleotides to evoke arterial contraction, α, β-mATP ≫ ATP > UTP indicates that stimulation of P_{2X} purinoceptor subtype predominantly mediates contraction (Windscheif et al., 1994). This suggestion is also supported by the finding that the ATP (1000 and 3000 nmol)-induced arterial contraction was attenuated to some degree by suramin, a P_2 purinoceptor antagonist (Dunn and Blakeley, 1988), and markedly and equi-effectively by PPADS, a P_{2X} purinoceptor antagonist (Lambrecht et al., 1992; Ziganshin et al., 1993) and α, β-mATP, a selective P_{2X} (currently designated $P2X_1$) purinoceptor agonist which desensitizes the receptors (Kasakov and Burnstock, 1983; Surprenant, 1996). Although PPADS may not necessarily act as a selective P_{2X} purinoceptor antagonist (Brown et al., 1995; Ralevic and Burnstock, 1996), in the present experiments, the agent almost completely inhibited the P_{2X} purinoceptor-mediated contraction, since the inhibition was equi-potent with that by α, β-mATP. Moreover, the response could not be further attenuated, even by the combination of PPADS, α, β-mATP and suramin (data not shown). The residual response, which was tolerant to the antagonists, appeared to be evoked through a receptor subtype other than P_{2X} and P_{2Y} purinoceptors, probably P_{2U} purinoceptors. Actually, the arterial UTP response was not attenuated by the antagonists, except for the weak inhibition by suramin, and further attenuation of the response by the combination of the antagonists was also not observed (data not shown). The augmentation of the arterial ATP response by reactive blue 2 suggests that the endothelial P_{2Y} purinoceptor-mediated vasodilation (Burnstock and Warland, 1987) regulates the constriction.

With the veins, dose-response curves for ATP and UTP were superimposed, while α, β-mATP failed to cause responses. Thus, in rat mesenteric veins, there are almost no functional P_{2X} ($P2X_1$) purinoceptors which give rise to vascular constriction. This can be compared with a previously reported finding in a [^{3}H] α, β-mATP binding study which showed sparse distribution of $P2X_1$ purinoceptors in the veins (Bo and Burnstock, 1993). On the other hand, the P_{2Y} purinoceptor-related relaxation has no role in the venous responses to ATP and UTP, since reactive blue 2 failed to change the responses.

It has been reported that, in the mesenteric arterial bed of rats, UTP constriction is resistant to PPADS (Windscheif et al., 1994), is resistant to both PPADS and suramin in the perfused pulmonary bed (Rubino and Burnstock, 1996), or resistant to PPADS but slightly inhibited by suramin in tail artery strips (McLaren et al., 1998). In the present study, the venous ATP response, as well as the UTP response, were resistant to PPADS and slightly inhibited by suramin. Thus, the venous ATP response seems to be mediated by stimulation at P_{2U} purinoceptors, that is, the $P2Y_2$ receptor subtype in the recent nomenclature for P2 receptors, where ATP and UTP are equi-active and PPADS has no effect, but suramin acts as a low-potency antagonist (Charlton et al., 1996a,b).

Another interesting finding was the enhancement of the arterial response to UTP by PPADS. It is not clear whether the potentiation was due to a direct action of PPADS on P_{2U} purinoceptors or the result of an indirect event which

involves receptor subtypes other than P_{2U} purinoceptors. However, a direct action on P_{2U} purinoceptors is unlikely, since the modification was not found in the veins, but in the arteries where P_{2X}, P_{2Y} and P_{2U} purinoceptor subtypes appear to be distributed. Also, inhibition of ecto-ATPase by the antagonist cannot be excluded, since PPADS and suramin have been reported to inhibit the enzyme, and PPADS especially was effective at 30 μM (Khakh et al., 1995).

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