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European Journal of Pharmacology 334 (1997) 173–180

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Pharmacological analysis of responses to ATP in the isolated and perfused canine coronary artery

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Received 20 March 1997; revised 25 June 1997; accepted 15 July 1997

Abstract

Vascular responses of the isolated and perfused canine coronary artery to adenosine 5′-triphosphate (ATP) were analyzed pharmacologically. At basal perfusion pressure, ATP induced a vasoconstriction followed by a vasodilation dose-dependently. The potency order for vasoconstriction was α,β-methylene ATP > 2-methylthio ATP > UTP > ATP. That for vasodilation was ATP > 2-methylthio ATP > α,β-methylene ATP ≫ UTP in the preparations precontracted by 20 mM KCl. Aminophylline inhibited the vasodilation induced by adenosine, but not that induced by ATP. α,β-Methylene ATP and suramin inhibited the vasoconstriction induced by ATP. Reactive blue 2 inhibited the vasodilation induced by ATP, but not the vasoconstriction. Removal of the endothelium by saponin and L-N^G-nitroarginine inhibited the vasodilation induced by ATP, but indomethacin did not. The results suggest that ATP induces vasoconstriction via P_{2X} purinoceptors on the smooth muscle and vasodilation via P_{2Y} purinoceptors on the endothelium through mainly the release of nitric oxide in the canine coronary artery, respectively. © 1997 Elsevier Science B.V.

Keywords: Coronary artery, canine; Vasoconstriction; Vasodilation; P_2 purinoceptor; ATP; UTP; Suramin; Reactive blue 2

1. Introduction

Adenosine 5′-triphosphate (ATP) is released as a cotransmitter with noradrenaline from perivascular sympathetic nerves and as a transmitter from purinergic and sensory nerves (Burnstock, 1972). The effects of extracellular ATP have been described in many physiological and pathophysiological processes: contractile responses of cardiac, vascular and visceral smooth muscle; stimulatory and inhibitory effects on neurons; stimulatory and inhibitory effects on ion; hormone; exocrine glands; platelets; mast cells; and many other effects (Harden et al., 1995). Although pharmacological effects of ATP were acknowledged as early as in 1929 (Drury and Szent-Györgyi, 1929), the effects of ATP have only received major attention only in the last 25 years since Burnstock reported the role of ATP as a cotransmitter (Burnstock, 1972). In the cardiovascular system, ATP is a component of platelets and erythrocytes and is released from endothelial and smooth muscle cells by ischemia, hypoxia and shear stress. Extracellular ATP plays an important role in the interaction between platelets and the vessel wall, mainly through the stimulation of prostacyclin and nitric oxide (NO) release from vascular endothelial cells (Gordon, 1986; Boeynaems and Pearson, 1990).

The receptors mediating responses to purines have been categorized in two types, P_1 and P_2, according to the selectivity for adenosine and ATP (Burnstock, 1978). P_2 purinoceptors have been subdivided into two major classes: ligand-gated P_{2X} and G-protein coupled P_{2Y} purinoceptors (Abbracchio and Burnstock, 1994). P_2 purinoceptors were further subdivided to P_{2U}, P_{2T}, P_{2Z}, P_{2D} purinoceptors in addition to previously major two purinoceptors (Harden et al., 1995). The P_{2U} purinoceptors are widely distributed and are stimulated by both ATP and UTP (Dubyak and El-Moatassium, 1993). On the other side, several differences between the effects of UTP and ATP led to a proposal for the existence of specific pyrimidinoceptors distinct from purinoceptors (Von Kügelgen et al., 1987). Recently, P2 receptors (7 and 8 subtypes of P_{2X} and P_{2Y} receptors, respectively) have been cloned and a new nomenclature of P2 receptors was proposed based on the structure and the signal transduction system (Fredholm et al., 1997). P_{2X} and P_{2Y} receptors are ligand-gated ion

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channels (Brake et al., 1994; Valera et al., 1994) and G-protein-coupled receptors (Boarder et al., 1995), respectively. P_{2X} purinoceptors were proposed to mediate contractile effects of ATP on smooth muscle. P_{2Y} purinoceptors were proposed to mediate the relaxant effects of ATP on smooth muscle and/or endothelium. There are many different reports about the locations of P_{2X} and P_{2Y} purinoceptors in the same species and vasculatures. In the rabbit mesenteric artery (Mathieson and Burnstock, 1985) and the rabbit portal vein (Kennedy and Burnstock, 1985), P_{2X} and P_{2Y} purinoceptors located on the smooth muscles. In the rabbit coronary artery (Corr and Burnstock, 1994), P_{2Y} purinoceptors are located on both the smooth muscles and the endothelium. In the rat mesenteric artery (Ralevic and Burnstock, 1988), P_{2X} and P_{2Y} purinoceptors are located on the smooth muscles and the endothelium, respectively. P_{2U} purinoceptors also mediate the vasoconstriction and the vasodilation induced by ATP (O'Connor et al., 1991).

ATP induced both coronary vasodilation and vasoconstriction in rat (Hopwood and Burnstock, 1987), rabbit (Corr and Burnstock, 1991, 1994) and guinea-pig (Vials and Burnstock, 1994). ATP induced vasoconstriction in the simian epicardial coronary artery (Nakane and Chiba, 1990). On the other hand, ATP induced only a vasodilation or a vasodilation followed by a rebound vasoconstriction in the canine coronary artery (Hashimoto et al., 1964; Houston et al., 1987; White and Angus, 1987). In view of the clinical prevalence of coronary vasospasm that affects primarily the large coronary arteries (Maseri et al., 1978), it is important to investigate the characteristics of purinoceptors of epicardial coronary arteries. In our preliminary studies, ATP induced a vasoconstriction followed by a vasodilation in the canine coronary artery and responses were not changed even in the coronary artery precontracted by 20 mM KCl. Therefore, the aim of the present study was to investigate the possible mechanisms of the responses of the canine coronary artery to ATP using purinoceptor antagonists and inhibitors of enzymatic synthesis of NO and prostacyclin.

2. Methods

2.1. Arterial preparations

Mongrel dogs (7–18 kg) of either sex were anesthetized with sodium pentobarbital (30 mg/kg, i.v.). After treatment with sodium heparin (200 U/kg, i.v.), the animals were sacrificed by rapid exsanguination. The heart was rapidly removed. The circumflex branch of the left coronary artery and right coronary artery, being superficially located, were removed from the heart and cleaned of loose adipose and connective tissues in cold Krebs–Henseleit solution. The arteries were cut into segments (1.0–2.4 mm outer diameter (O.D.) and 1.5 cm long). All side branches were tied with silk thread. A segment was carefully cannulated with a stainless steel needle type cannula (0.6–2.65 mm O.D.). The cannulated arterial segment was placed in a cup-shaped glass bath and was perfused by a peristaltic pump (Tokyo Rikakikai, MP300) with Krebs–Henseleit solution gassed with 95% O_2 and 5% CO_2. The composition of Krebs–Henseleit solution was (mM): NaCl 118, KCl 4.7, $MgSO_4$ 1.2, $CaCl_2$ 2.5, KH_2PO_4 1.2, $NaHCO_3$ 25 and glucose 10. The flow rate was kept at about 1.2 ml/min. Perfusion pressure was measured with an electric manometer (Nihon Kohden, AP 621G) and recorded with a rectigraph (Nihon Kohden, WT-685H). The basal perfusion pressure was 40–100 mm Hg. Vasoconstriction or vasodilation was recorded as an increase or a decrease in perfusion pressure, respectively. For the pharmacological analysis of vasodilation, the concentrations of NaCl and KCl in Krebs–Henseleit solution were changed to (mM): 102.7 and 20, respectively. The perfusion pressure was raised to 80–200 mm Hg. After 1 h equilibration, agonists were administered into the rubber tube connecting with the cannula in a volume of 0.01–0.03 ml by a microinjector (Terumo, Tokyo) and the injection time was approximately 4 s. Antagonists and inhibitors were dissolved in perfusate. The preparations were tested for the presence or absence of the endothelium by acetylcholine. In the preliminary experiments, the responses of the left circumflex coronary artery to agonists were not different from those of the right coronary artery. To remove endothelium, saponin (1 mg) was injected to the rubber tube connecting with the cannula in a volume of 0.01 ml for 4 s as described previously (Nakane et al., 1986). After the saponin injection, the perfusion pressure greatly increased. The increased perfusion pressure gradually decreased and reached the previous baseline after about 90 min. Then, agonists were tested.

2.2. Drugs

Drugs used were: adenosine 5′-triphosphate (ATP, Sigma, USA); α,β-methylene adenosine 5′-triphosphate (Sigma); 2-methylthioadenosine 5′-triphosphate (Research Biochemicals, USA); uridine 5′-triphosphate trisodium salt (UTP, Sigma); acetylcholine chloride (Daiichi Pharmaceutical, Japan); adenosine (Tokyo Kasei Kogyo, Japan); aminophylline (Eisai, Japan); reactive blue 2 (RBI); suramin sodium (Wako Pure Chemical, Japan); saponin (Merck, Germany); papaverine hydrochloride (Dainippon Pharmaceutical, Japan); L-N^G-nitroarginine (L-NOARG, RBI); indomethacin (Wako). Stock solutions of indomethacin and L-NOARG were made up in ethanol and 0.1 N HCl, respectively. Other drugs were dissolved in physiological saline.

2.3. Statistical analysis

Vascular responses to drugs were expressed as the maximal changes in perfusion pressure (mm Hg) from

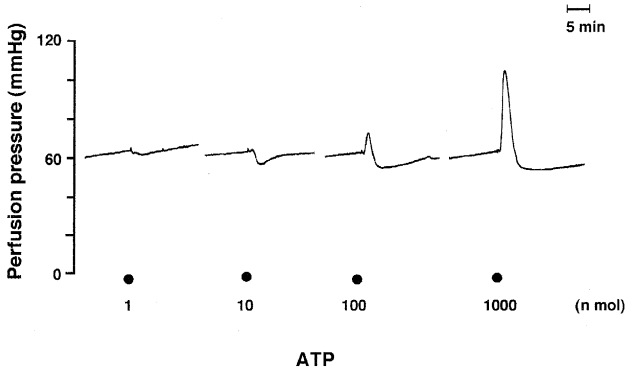

Fig. 1. Responses of the isolated and perfused canine coronary artery to ATP.

their control levels. Values presented in the text and figures are the means ± S.E.M. Since some of the agonists did not reach to the maximal response, ED_{50} values could not be calculated. Therefore, the potency order of agonists was evaluated empirically by comparing the dose–response curves obtained. Two-way analyses of variance and Bonnferroni–Dunn test were used to evaluate the data. If the statistic value was significant, we evaluated statistical significance by Student's *t* test for paired data. *P* values less than 0.05 were statistically considered significant.

3. Results

3.1. Responses of the canine coronary artery to ATP, 2-methylthio ATP, α,β-methylene ATP and UTP

At basal perfusion pressure, ATP induced a biphasic and dose-related response that was a brief vasoconstriction followed by a long-lasting vasodilation (Fig. 1). 2-Methylthio ATP and UTP elicited a vasoconstriction in a dose-related manner. α,β-Methylene ATP induced a vasoconstriction at 10^{-11}–3×10^{-9} mol in a dose-related manner and reached the plateau at 10^{-8} mol as shown in Fig. 2. The same consecutive dose (10^{-11}–3×10^{-9} mol) of α,β-methylene ATP injection did not induce desensitization (5 min interval, 3 times; data not shown). The rank order of potency for vasoconstriction was α,β-methylene ATP > 2-methylthio ATP > UTP > ATP (Fig. 2). Because each vasodilation was small at basal perfusion pressure, we evaluated the vasodilation in the preparation precontracted by 20 mM KCl. These agonists caused a dose-dependent vasodilation with the potency order of ATP > 2-methylthio ATP > α,β-methylene ATP ≫ UTP (Fig. 2). The responses to α,β-methylene ATP were small and only a high dose of UTP induced vasodilation.

3.2. Effects of aminophylline on vasodilator responses to adenosine and ATP

To determine whether ATP acts via the breakdown to adenosine, we examined the effects of a P_1 purinoceptor antagonist aminophylline on the vasodilator responses to adenosine and ATP. In the precontracted preparations, the perfusion with aminophylline (10 μM) inhibited the vasodilator responses to adenosine (10^{-9}–10^{-7} mol) as previously reported (Nakane and Chiba, 1993). Vasodilator responses to ATP were slightly reduced to lower doses (10^{-9}–10^{-7} mol), but not significant (Fig. 3). Aminophylline did not affect the vasoconstrictions induced by ATP (Fig. 3).

3.3. Effects of the P_{2X} purinoceptor desensitization with α,β-methylene ATP, reactive blue 2 and suramin on the responses to ATP

To determine whether ATP acts via P_{2X} purinoceptors, we examined the effect of the $P2X_1$ and $P2X_3$ purinocep-

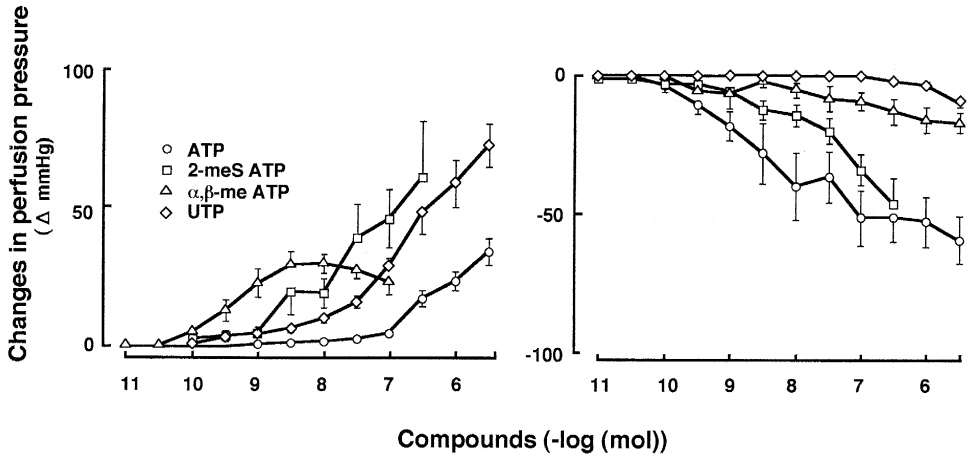

Fig. 2. Responses of the isolated and perfused canine coronary artery to ATP, 2-methylthio ATP (2-meSATP), α,β-methylene ATP (α,β-meATP) and UTP. Vasodilations were examined in the raised perfusion pressure by 20 mM KCl. Data represent the mean ± S.E. of the indicated number of experiments. For vasoconstriction: ATP ($n = 14$), 2-methylthioATP ($n = 6$), α,β-methylene ATP ($n = 8$) and UTP ($n = 10$). For vasodilation: each 12 experiments.

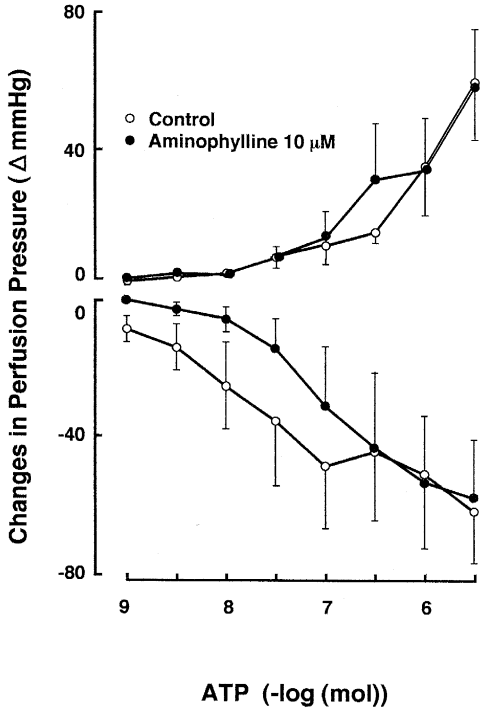

Fig. 3. Effects of aminophylline (10 μM) on the responses of the isolated and perfused canine coronary artery precontracted by 20 mM KCl to ATP. Data represent the mean ± S.E. of 6 experiments.

tor desensitizing agent α,β-methylene ATP on the vasoconstrictor responses to ATP. The perfusion with α,β-methylene ATP (1 μM) initially induced a great increase in perfusion pressure. The increased perfusion pressure gradually decreased and reached the previous baseline after about 1 h. The perfusion with α,β-methylene ATP (1 μM) reduced the vasoconstrictor responses to ATP (Fig. 4), but α,β-methylene ATP (3 μM) did not significantly affect vasodilations induced by ATP (Fig. 4). The perfusion with reactive blue 2, a P_2 purinoceptor antagonist (10

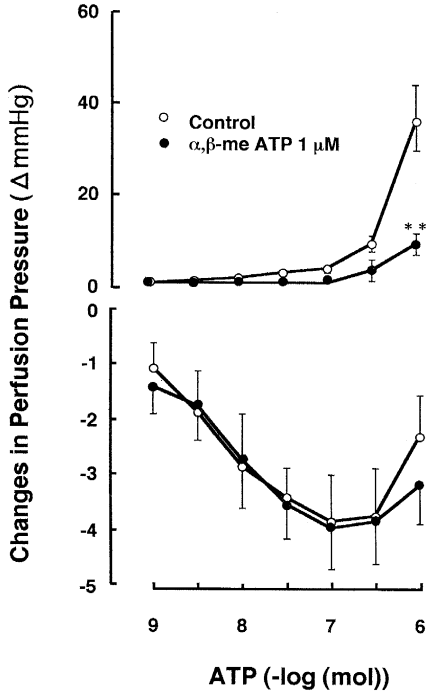

Fig. 4. Effects of the P_{2X} purinoceptor desensitization with α,β-methylene ATP (α,β-meATP, 1 μM) on the responses of the isolated and perfused canine coronary artery to ATP. Data represent the mean ± S.E. of 10 experiments. $^{**}P < 0.01$ compared to controls.

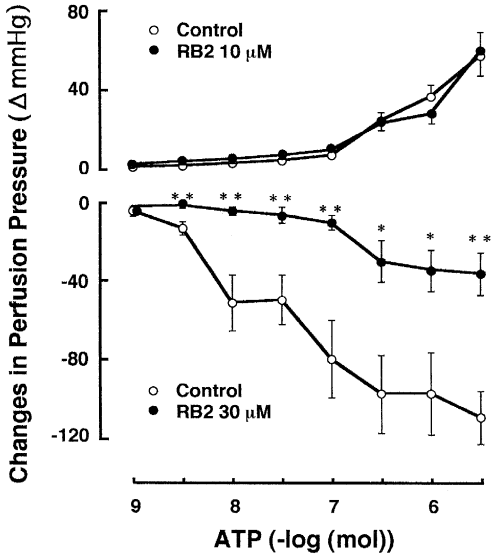

Fig. 5. Effects of reactive blue 2 (RB2, 10 μM for vasoconstriction; 30 μM for vasodilation) on the responses of the isolated and perfused canine coronary artery to ATP. Vasodilations were examined in the raised perfusion pressure by 20 mM KCl. Data represent the mean ± S.E. of 6 experiments. $^{*}P < 0.05$ and $^{**}P < 0.01$ compared to controls.

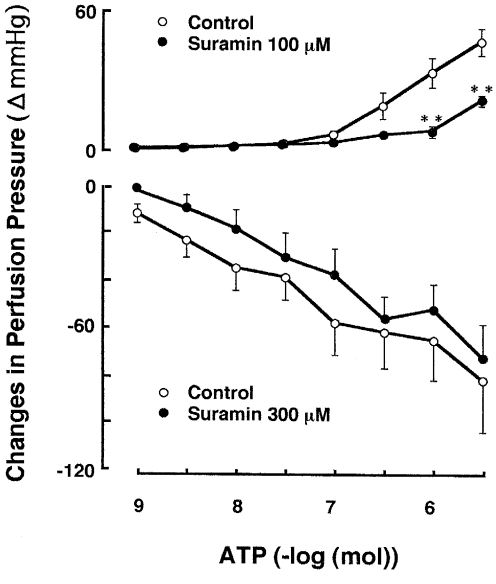

Fig. 6. Effects of suramin (100 μM for vasoconstriction; 300 μM for vasodilation) on the responses of the isolated and perfused canine coronary artery to ATP. Vasodilations were examined in the raised perfusion pressure by 20 mM KCl. Data represent the mean ± S.E. of the responses of 7 experiments. $^{*}P < 0.01$ compared to controls.

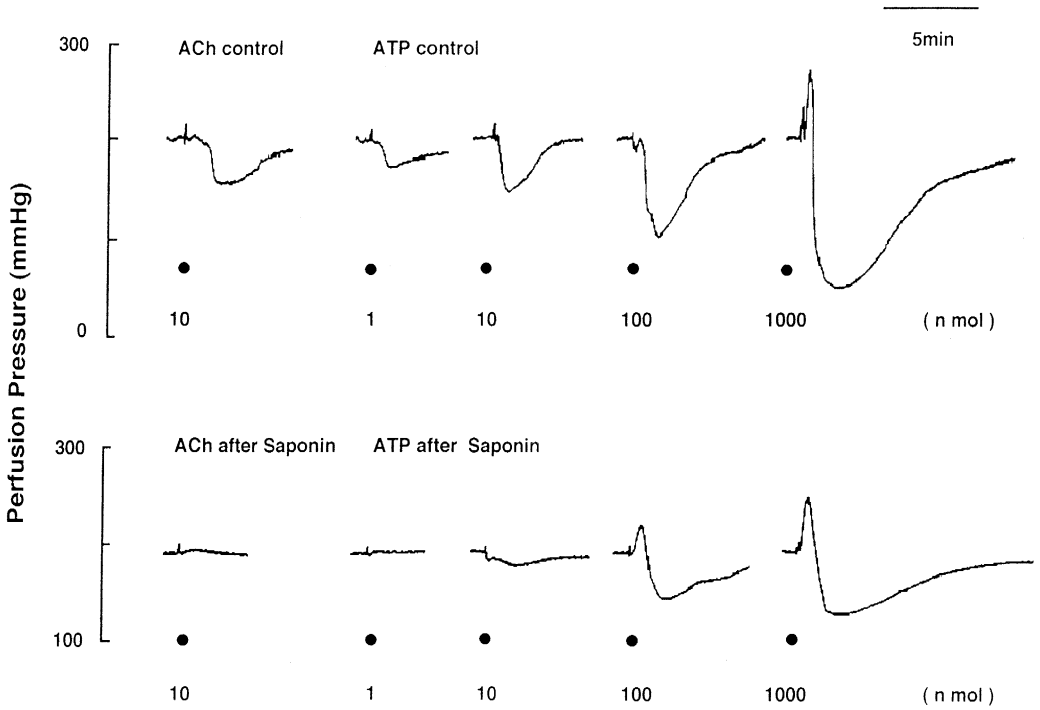

Fig. 7. Responses of the isolated and perfused canine coronary artery precontracted by 20 mM KCl to ATP (10^{-9}–3×10^{-6} mol) and acetylcholine (10^{-8} mol).

μM), did not affect the ATP-induced vasoconstrictions, but 30 μM reactive blue 2 inhibited ATP-induced vasodilations in the precontracted preparations (Fig. 5). The perfusion with suramin, a non-selective P_2 purinoceptor antagonist (100 μM), inhibited the vasoconstriction induced by ATP (Fig. 6). On the other hand, suramin (100 and 300 μM) slightly reduced ATP-induced vasodilations, but not significant (Fig. 6).

3.4. Effects of the endothelium removal on the responses to ATP

The removal of the endothelium by saponin (1 mg) reduced the vasodilator responses to acetylcholine, but it did not affect those to papaverine (1 μmol) as previously reported (Nakane et al., 1986). The removal of the endothelium inhibited the vasodilations induced by ATP, although it did not affect the vasoconstrictions (Figs. 7 and 8).

3.5. Effects of L-NOARG and indomethacin on the responses to ATP

We examined the effects of L-NOARG, a selective NO synthase inhibitor, and indomethacin, a cyclooxgenase inhibitor, on the responses to ATP. L-NOARG (300 μM) significantly reduced the vasodilator responses to ATP (Fig. 9a). The perfusion with indomethacin (1 μM) initially induced a great increase in perfusion pressure (data not shown). The increased perfusion pressure gradually decreased, but it did not reach the previous baseline after 90 min. Thus, in this study, vasodilator responses are presented as the percentage of the vasodilator responses to

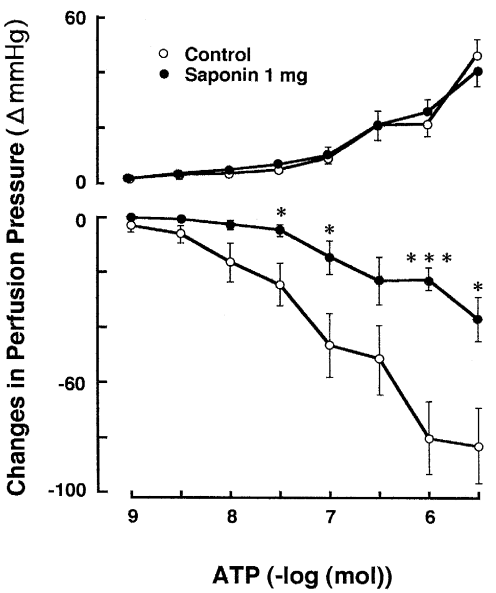

Fig. 8. Effects of intraluminal saponin (1 mg) on the responses of the isolated and perfused canine coronary artery precontracted by 20 mM KCl to ATP. Data represent the mean ± S.E. of 9 experiments. $^{*}P < 0.05$ and $^{***}P < 0.001$ compared to controls.

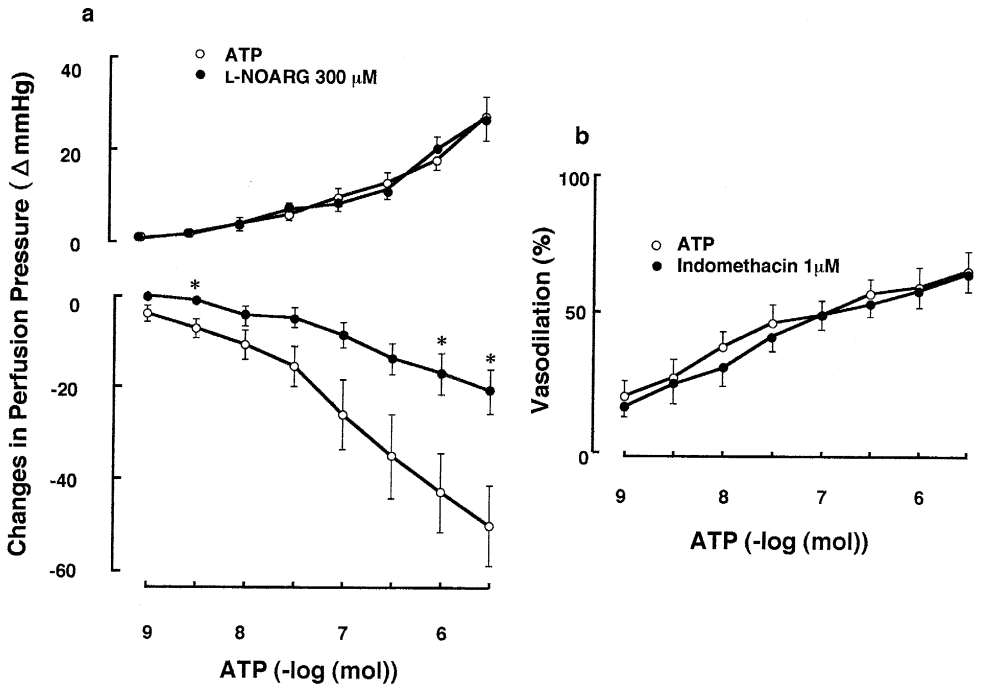

Fig. 9. Effects of L-NOARG (a: 300 μM) and indomethacin (b: 1 μM) on the responses of the isolated and perfused canine coronary artery precontracted by 20 mM KCl to ATP. Data represent the mean ± S.E. of 7 experiments. Vasodilator responses to 1 μmol papaverine were considered as 100%. $^{*}P < 0.05$ compared to controls.

papaverine 1 μmol (Nakane et al., 1988). Indomethacin did not affect the vasodilator responses to ATP (Fig. 9b).

4. Discussion

The pharmacological analysis of the responses to ATP in the canine coronary artery suggests that the initial vasoconstriction and the subsequent vasodilation are mediated by P_{2X} purinoceptors on the smooth muscle and P_{2Y} purinoceptors mainly on the endothelium, respectively. ATP induces a vasodilation mainly through NO release, but prostacyclin appears to play a minimal role.

In this study, ATP induced a dose-dependent vasoconstriction followed by a vasodilation in the canine coronary artery. The vasoconstrictor responses of the canine coronary artery to ATP were inhibited by α,β-methylene ATP that desensitizes $P2X_1$ and $P2X_3$ receptors of the new nomenclature (Fredholm et al., 1997) and the non-selective P_2 purinoceptor antagonist suramin (Hoyle et al., 1990). In the isolated rabbit coronary artery, ATP induced transient vasoconstriction mediating via P_{2X} purinoceptors on the smooth muscle (Corr and Burnstock, 1994). Thus, P_{2X} purinoceptors ($P2X_1$ and/or $P2X_3$ receptors), that are ligand-gated ion channels, mediate the vasoconstriction induced by ATP in the canine coronary artery.

α,β-Methylene ATP induced a vasoconstriction at 10^{-11} mol, but it did not reach the maximum responses to other agonists even at high doses. To examine the effects of desensitization with α,β-methylene ATP itself, the preparations were tested by the repeated injections of the same dose (10^{-11}–10^{-8} mol, 5 min interval, 3 times) and by the lowest dose (10^{-11} mol) injection after the highest dose (10^{-7} mol) injection. The repeated injections of α,β-methylene ATP (10^{-11}–3×10^{-9} mol) did not induce desensitization, but the responses to α,β-methylene ATP (10^{-11} mol) were inhibited after the injection of α,β-methylene ATP 10^{-7} mol. The results suggests that the responses to α,β-methylene ATP were inhibited by its desensitization effect at higher doses (more than 3×10^{-9} mol).

ATP induced a marked vasodilation in the canine coronary artery precontracted by 20 mM KCl. The rank order of potency for vasodilation was ATP > 2-methylthio ATP > α,β-methylene ATP $\gg$ UTP in this study, but it did not correspond to those for the P_{2Y} (Burnstock and Kennedy, 1985) and the P_{2U} (O'Connor et al., 1991) purinoceptors that are $P2Y_1$ and $P2Y_2$ receptors in the new nomenclature (Fredholm et al., 1997), respectively. P2Y receptors are G-protein-coupled seven transmembrane receptors. Only $P2Y_1$ receptors are insensitive to UTP. ATP and UTP are equipotent to $P2Y_2$ receptors. The perfusion with α,β-methylene ATP did not affect the vasodilator responses to ATP (Fig. 4). Reactive blue 2 inhibited the vasodilator responses to ATP (Fig. 5). Reactive blue 2 antagonizes P_{2Y} purinoceptors, but a low concentration of reactive blue 2 was reported to have antagonist activity to P_{2X} purinoceptor (Kennedy and Leff, 1995). Reactive blue 2 did not affect the vasoconstriction induced by ATP that is mediated by P_{2X} purinoceptors in this study. Suramin did not

inhibit the vasodilator responses of the canine coronary artery to ATP, although it inhibited the vasoconstrictor responses. Suramin may be more effective as an antagonist to P_{2X} purinoceptors than P_{2Y} purinoceptors (Hoyle et al., 1990; Leff et al., 1990; O'Connor et al., 1991). Thus, P_{2Y} purinoceptors ($P2Y_1$ receptors) mediate the vasodilator responses of the canine coronary artery to ATP. This is the same as found in the previous report (Houston et al., 1987).

Some ATP actions are mediated by its breakdown product, adenosine, via P_1 purinoceptors (Hopwood and Burnstock, 1987, Corr and Burnstock, 1991). In this study, the inhibitory effect of aminophylline (a P_1 purinoceptor antagonist) on the vasodilator responses to ATP was not statistically significant, although aminophylline seems to reduce those to low doses of ATP (1–10 nmol) as shown in Fig. 3. Reactive blue 2 (30 μM) inhibited the vasodilator responses to ATP (Fig. 8). Reactive blue 2 (1.8 μM) reduced partially the responses to adenosine in rat Langendorff preparations (Hopwood and Burnstock, 1987), but 10 μM of it did not affect those in rat mesenteric artery rings (Vuorinen et al., 1994). The removal of endothelium by saponin and L-NOARG inhibited the vasodilator responses to ATP in this study. Adenosine-induced vasodilation is not endothelium-dependent (Furchgott, 1984; Nakane and Chiba, 1993). Thus, it seems that the breakdown of ATP to adenosine does not play an important role in the vasodilation induced by ATP in the canine coronary artery.

In this study, ATP induced a vasoconstriction, although the endothelium was functionally intact. ATP induced only endothelium-dependent vasodilations in ring preparations of the canine coronary artery (Houston et al., 1987; White and Angus, 1987). The removal of the endothelium by 1 mg saponin inhibited the vasodilation induced by ATP, but it did not affect the vasoconstriction. The smooth muscle was intact after saponin treatment in this study, because the vasodilator responses to papaverine were not changed. We showed that saponin (1 mg) treatment selectively removed endothelium of the canine epicardial coronary artery using light and electron microscopies (Nakane et al., 1986). The removal of the endothelium inhibited the ATP-induced vasodilations, but it did not abolish it. ATP elicited vasodilations by a direct action on the smooth muscle in the rabbit coronary artery (Corr and Burnstock, 1991, 1994). ATP acts via P_{2Y} purinoceptors on the endothelium in the rat mesenteric artery (Ralevic and Burnstock, 1988) and in the canine coronary artery (this study). Thus, ATP induces the vasoconstriction and the vasodilation via P_{2X} purinoceptors on the smooth muscle and P_{2Y} purinoceptors on the endothelium of the canine coronary artery, respectively.

Vials and Burnstock (1993) reported that the vasodilations induced by ATP appear to involve prostacyclin and NO in the guinea-pig coronary arteries. In this study, L-NOARG, an NO synthase inhibitor (Moore et al., 1990) like L-N^G-monomethyl arginine, inhibited the vasodilations induced by ATP, but indomethacin, a cyclooxygenase inhibitor, did not affect those. Therefore, a major part of the vasodilation induced by ATP is via production of NO, and prostanoids appear to play a minimal role in the canine coronary artery.

UTP induced a dose-dependent vasoconstriction and only a high dose of UTP induced a slight vasodilation in the precontracted preparations (Fig. 2). There are a few reports about the response of the coronary artery to UTP. UTP induced only a vasodilation in the canine coronary artery (Hashimoto et al., 1964) and the guinea-pig coronary artery (Vials and Burnstock, 1993). They observed the responses of the coronary artery to UTP in the Langendorff preparations. Thus, this difference is based on the preparations of the different methods. UTP was proposed to act via P_{2U} purinoceptors on the smooth muscle and endothelium that mediates vasoconstriction and vasodilation, respectively (O'Connor et al., 1991). On the other hand, several differences between the effects of UTP and ATP have led to a proposal for the existence of specific pyrimidinoceptors, distinct from purinoceptors (Von Kügelgen et al., 1987). Cloned $P2Y_2$, $P2Y_4$ and $P2Y_6$ receptors have high affinities with UTP (Charlton et al., 1996; Nicholas et al., 1996). $P2Y_2$ receptors (P_{2U} purinoceptors) respond to both ATP and UTP equipotently. $P2Y_4$ and $P2Y_6$ receptors are selective for UTP and UDP after blocking of nucleotide diphosphokinases, respectively. Further studies are needed to confirm which receptor mediates UTP action in the canine coronary artery.

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