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UTP induces vascular responses in the isolated and perfused canine epicardial coronary artery via UTP-preferring P2Y receptors

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1 Vasoconstrictor responses of the isolated and perfused canine epicardial coronary artery to uridine 5′-triphosphate (UTP) were analysed pharmacologically.

2 At basal perfusion pressure, UTP induced vasoconstriction in a dose-related manner and the vasoconstriction was sometimes followed by a slight vasodilatation at large doses (more than 10 nmol). The rank order of potency for vasoconstriction was UTP = UDP > ATP > TTP ≥ ITP > > UMP. At raised perfusion pressure by 20 mM KCl, the vasoconstriction was not changed and a small vasodilatation was induced at large doses. The rank order of potency for vasodilatation was induced at large doses. The rank order of potency for vasodilatation was ATP > > ITP ≥ UDP > UTP ≥ TTP. The maximal vasodilator response to UTP was much less than that to ATP. UMP did not induce vasodilatation.

3 The P2X receptor agonist and desensitizing agent α,β-methylene ATP (1 μM) and the P2 receptor antagonist suramin (100 μM) inhibited the vasoconstrictor responses to ATP but not those to UTP and UDP. The P2 receptor antagonist reactive blue 2 (30 μM) did not inhibit the vascular responses to UTP.

4 UTP (200 μM) desensitized the vasoconstrictor responses to UTP, but not either the vasodilator responses to UTP or the vasoconstrictor responses to ATP and UDP. UDP (200 μM) did not desensitize the vascular responses to UTP.

5 Preincubating the UDP stock solution and arterial preparation with hexokinase (10 and 1 uml^{-1}, respectively) did not change the vasoconstrictor responses to UDP.

6 The Ca channel blocker diltiazem (1 μM) inhibited the vasoconstrictor responses to UTP but not those to ATP and UDP. Incubation in a Ca^{2+}-free solution containing 1 mM EGTA inhibited the vascular responses to ATP, UTP and UDP.

7 Removal of the endothelium by an intraluminal injection of saponin (1 mg) inhibited the vasodilator responses to UTP. Indomethacin, a cyclo-oxygenase inhibitor (1 μM), inhibited the vasodilator responses to UTP, but N^G-nitro-L-arginine, a nitric oxide synthase inhibitor (300 μM), did not have an inhibitory effect.

8 The results suggest that (1) UTP induces vasoconstriction via UTP-preferring P2Y receptors on the smooth muscle and vasodilatation via receptors different from those mediating the vasoconstriction induced by UTP and mediating the vasodilatation by ATP on the endothelium, through mainly the release of prostacyclin in the canine epicardial coronary artery; (2) UDP induces vasoconstriction via UDP-preferring P2Y receptors; and (3) L-type Ca ion channels are involved in the vasoconstriction induced by UTP, but not in that induced by UDP.

Keywords: UTP; UDP; P2 receptors; canine coronary artery; vasoconstriction; vasodilatation; ATP; suramin; nitric oxide; prostacyclin

Introduction

Uracil nucleotides are released from the granules of platelets and other organs into blood under a variety of pathological conditions such as trauma, hypoxia and inflammation (Goetz *et al*., 1971; Gordon, 1986; Seifert & Schultz, 1989). Uracil nucleotides may regulate the vascular tone and the blood coagulation similar to adenine nucleotides. As with adenosine 5′-triphosphate (ATP), both vasoconstrictor and vasodilator responses to uridine 5′-triphosphate (UTP) have been described (Seifert & Schultz, 1989). There are only a few studies on the vascular effects of uracil nucleotides on coronary circulation. UTP produced only vasodilatation or a small vasoconstrictor response followed by a large vasodilator response in the Langendorff preparations of the dog (Hashimoto *et al*., 1964) and the guinea pig (Vials & Burnstock, 1993), respectively. In our previous study (Matsumoto *et al*., 1997), UTP induced only vasoconstrictior vasoconstriction followed by a small vasodilator response in the canine epicardial coronary artery, although acetylcholine (ACh) induced vasodilatation. In view of the clinical prevalence of coronary vasospasm that affects primarily the large coronary arteries (Maseri *et al*., 1978), it is important to study the responses of epicardial coronary arteries to UTP.

UTP was proposed to act via P2U receptors and specific pyrimidinoceptors distinct from P2U receptors based on the results of physiological experiments (Häussinger *et al*., 1987; Von Kügelgen *et al*., 1987; Seifert & Schultz, 1989; Saïag *et al*., 1990; O'Connor *et al*., 1991). Recently, $P2Y_2$, $P2Y_4$ and $P2Y_6$ receptors that mediate some actions of UTP have been cloned (Lustig *et al*., 1993; Chang *et al*., 1995; Communi *et al*., 1995; 1996; Nguyen *et al*., 1995). These receptors belong to the superfamily of G-protein-coupled seven transmembrane domain receptors, are coupled to phospholipase C and are closely related to P2Y receptors that respond to ATP. $P2Y_2$ receptors recognize both ATP and UTP (Lustig *et al*., 1993; Nicholas *et al*., 1996). The potency order for stimulating $P2Y_2$ receptors was UTP = ATP > > 2-methylthio ATP = α,β-methylene ATP (Lustig *et al*., 1993). Nicholas *et al*. (1996) showed that $P2Y_4$ and $P2Y_6$ receptors are selectively activated by UTP and UDP, respectively. Fredholm *et al*. (1997) proposed the new nomenclature of P2 receptors based on the structure and the

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signal transduction system of the cloned receptors and recommended the use of the phrase, 'UTP-preferring P2Y receptors' in functional studies of the isolated tissues. P2X and P2Y receptors are ligand-gated ion channels and G-protein-coupled seven transmembrane domain receptors, respectively (Fredholm *et al*., 1997). α,β-Methylene ATP is a slowly degradable analogue of ATP which activates and desensitizes $P2X_1$ and $P2X_3$ receptors (Valera *et al*., 1994; Chen *et al*., 1995). α,β-Methylene ATP inhibited the actions of ATP, but not those of UTP in blood vessels (Von Kügelgen *et al*., 1987; Saïag *et al*., 1990; Von Kügelgen & Starke, 1990; Ralevic & Burnstock, 1991; García-Velasco *et al*., 1995). UTP desensitized the responses to UTP, but not those to ATP in blood vessels (Von Kügelgen *et al*., 1987; Saïag *et al*., 1990; Ralevic & Burnstock, 1991), the rat perfused liver (Häussinger *et al*., 1987) and the rat superior cervical ganglion (Connolly, 1994). Thus, it is hypothesized that UTP stimulates UTP-preferring P2Y receptors in some tissues.

The canine isolated and perfused epicardial coronary artery was used to study the possible mechanisms of the responses to UTP. Previous characterization showed that P2X and P2Y receptors were involved in the vasoconstrictor and vasodilator responses of canine epicardial coronary artery to ATP, respectively (Matsumoto *et al*., 1997). In this study, we characterized the receptors that are involved in the vascular responses of the canine epicardial coronary artery to UTP based on (1) the rank order of potency; (2) the effects of P2X receptor desensitization by α,β-methylene ATP and P2 receptor antagonists (reactive blue 2 and suramin); (3) the desensitizing effects of UTP and UDP; (4) the effect of hexokinase on the vasoconstrictor responses to UDP; (5) the effect of the Ca channel blocker, diltiazem, and removal of extracellular Ca^{2+}; and (6) the inhibitory effects of N^G-nitro-L-arginine (L-NOARG) and indomethacin on nitric oxide (NO) and prostacyclin formation.

Methods

Arterial preparations

Mongrel dogs (7–18 kg) of either sex were anaesthetized with sodium pentobarbitone (30 mg kg^{-1}, i.v.). After treatment with sodium heparin (200 ukg^{-1}, i.v.), the animals were killed 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^{-1}. 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 mmHg. Vasoconstriction or vasodilatation was recorded as an increase or a decrease in perfusion pressure, respectively. For the pharmacological analysis of vasodilatation, 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 mmHg. 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 Co., Tokyo) and the injection time was approximately 4 s. Antagonists and inhibitors were dissolved in perfusate and were tested after 1 h of perfusion. The preparations were tested for the presence or absence of the endothelium by ACh as previously described (Nakane *et al*., 1986). In the preliminary experiments, the responses of the left circumflex coronary artery to agonists were not different from those of the right coronary artery.

Drugs

Drugs used were: adenosine 5′-triphosphate (ATP); α,β-methylene adenosine 5′-triphosphate; uridine 5′-monophosphate

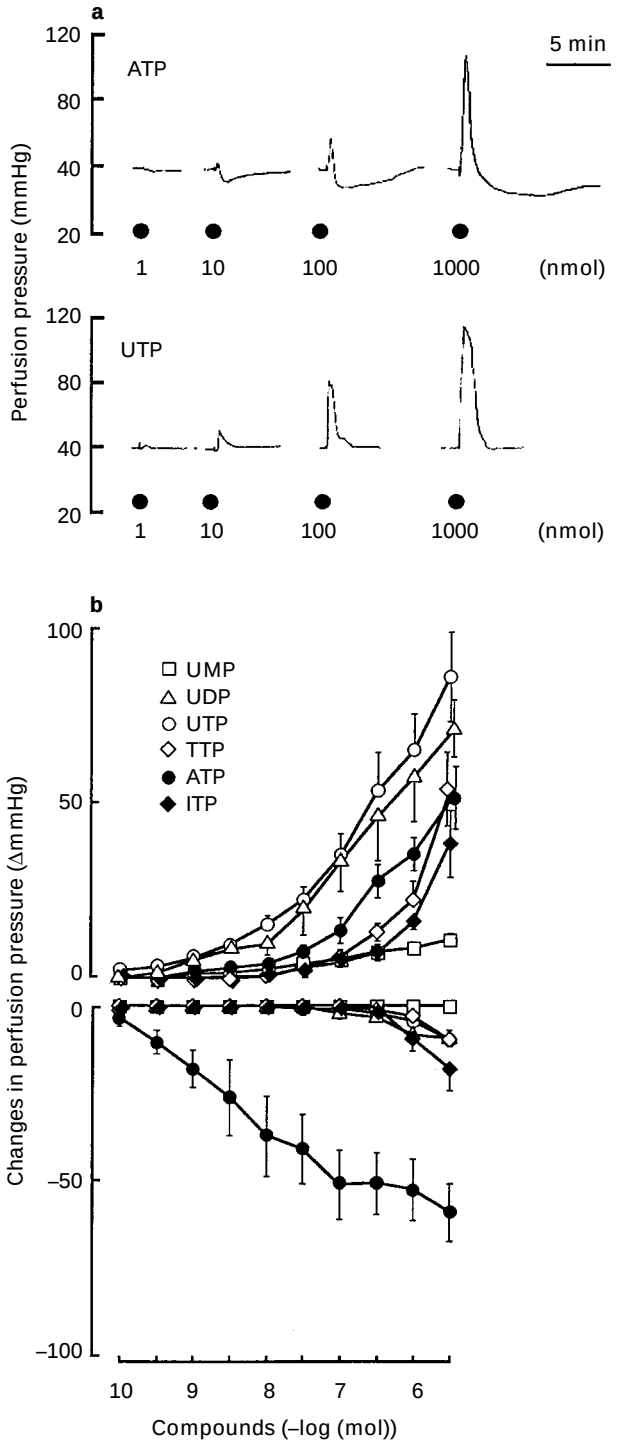

Figure 1 (a) Responses of the isolated and perfused epicardial canine coronary artery to ATP and UTP. (b) Dose-response curves of ATP, ITP, TTP, UTP, UDP and UMP for vasoconstriction and vasdilatation in the canine isolated and perfused epicardial coronary artery. Vasodilatations were induced after the perfusion pressure had been raised by 20 mM KCl. Each point represents the mean, and vertical lines show s.e.mean, of 6–12 of experiments.

trisodium salt (UMP); uridine 5′-diphosphate trisodium salt (UDP); uridine 5′-triphosphate trisodium salt (UTP); inosine 5′-triphosphate trisodium salt (ITP); thymidine 5′-triphosphate trisodium salt (TTP, all Sigma, St. Louis, U.S.A.); acetylcholine chloride (Daiichi Pharmaceutical Co, Tokyo, Japan); hexokinase (Boehringer-Mannheim Biochemicals, Mannheim, Germany), prostaglandin $F_{2\alpha}$ (Ono Pharmaceutical Co, Osaka, Japan); reactive blue 2 (Research Biochemicals International, Natick, U.S.A.); suramin sodium (Wako Pure Chemical Ind., Osaka, Japan); saponin (Merck, Darmstadt, Germany); diltiazem chloride (Tanabe Pharmaceutical Co, Osaka, Japan); papaverine hydrochloride (Dainippon Pharmaceutical Co, Tokyo, Japan); N^G-nitro-L-arginine (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.

Statistical analysis

Vascular responses to drugs were expressed as the maximal changes in perfusion pressure (mmHg) from their control levels. Values presented in the text and figures are the mean ± s.e.mean. Since a maximal response could not be obtained to some of the agonists, 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 statistical value was significant, we evaluated statistical significance by Student's *t* test for paired data. *P* values less than 0.05 were to be considered statistically significant.

Results

Responses of the canine coronary artery to nucleotides

At basal perfusion pressure, UTP elicited vasoconstriction in a dose-related manner (Figure 1a). High doses of UTP (more than 10 nmol) induced vasoconstriction followed by a small vasodilator response in 28 or 53 preparations. ATP induced a brief period of vasoconstriction followed by a long-lasting vasodilatation, as previously found (Matsumoto *et al*., 1997). The potency order for vasoconstriction was UTP = UDP > ATP > TTP ≥ ITP > > UMP (Figure 1b). Because the vasodilatation was small at basal perfusion pressure, we evaluated the vasodilatation after the perfusion pressure had been raised by 20 mM KCl. UTP induced a second phase vasodilatation in 28 of 48 precontracted preparations. These agonists caused dose-dependent vasodilatation with the potency order of ATP > > ITP ≥ UDP > UTP ≥ TTP (Figure 1b). The maximal vasodilator response to UTP was much less than that to ATP. UMP did not induce vasodilatation.

Effects of the P2X receptor desensitization with α,β-methylene ATP, reactive blue 2 and suramin on the responses to ATP, UTP and UDP

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 baseline after about 1 h. Perfusion with α,β-methylene ATP (1 μM) did not affect the vasoconstrictor responses to UTP and UDP (Figure 2b and c), although it inhibited those to ATP (Figure 2a). α,β-Methylene ATP (1 μM) did not affect the vasodilator responses to ATP and UTP (data not shown). Reactive blue 2 (30 μM) did not affect the vascular responses to UTP (data not shown). Suramin (100 μM) did not reduce the vasoconstrictor responses to UTP and UDP (Figure 2e and f), although it inhibited those to ATP (Figure 2d), but did not inhibit the vasodilator responses to ATP, UTP and UDP (data not shown).

Effects of UTP- and UDP-induced desensitization on the responses to ATP, UTP and UDP

UTP (200 μM) and UDP (200 μM) were perfused for 5 min and once the perfusion pressure had returned to the control level, ATP, UTP and UDP were applied. Perfusion with UTP

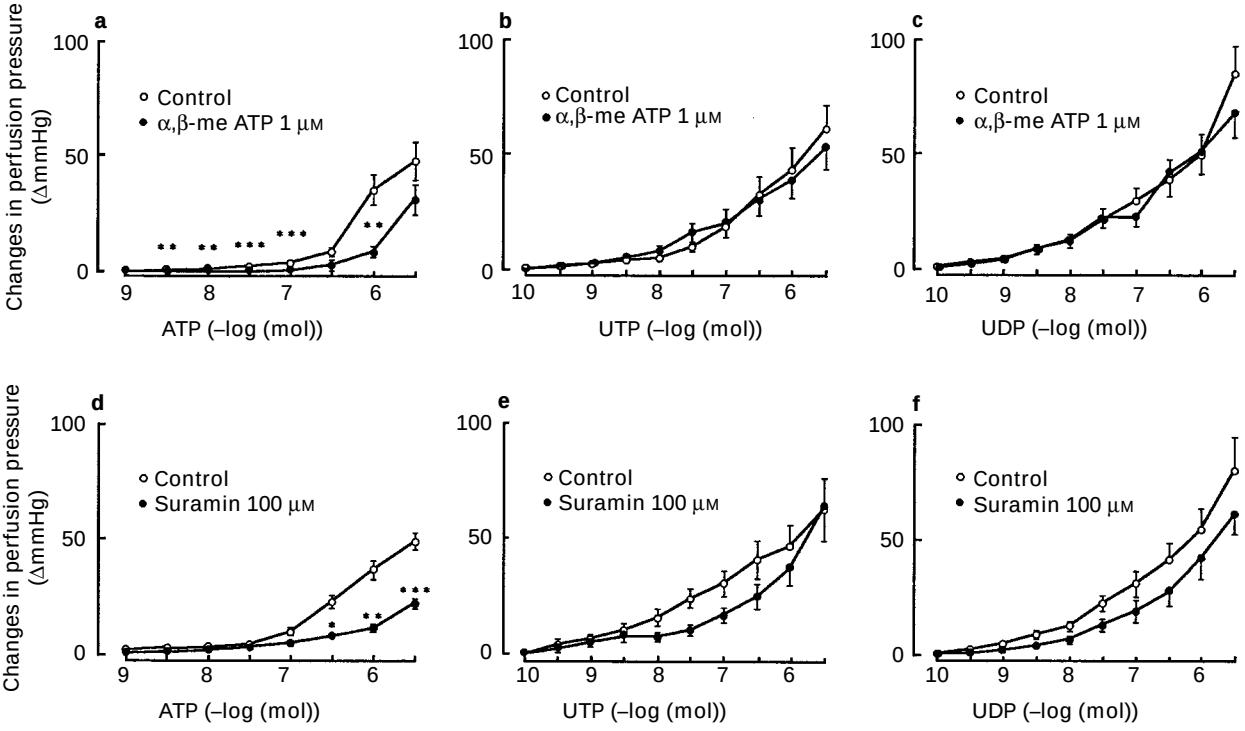

Figure 2 Effects of P2X-purinoceptor desensitization with α,β-methylene ATP (a–c; α,β-meATP, 1 μM) and suramin (d–f; 100 μM) on the vasoconstrictor responses of the canine isolated and perfused epicardial coronary artery to ATP, UTP and UDP. Each point represents the mean, and vertical lines show s.e.mean, of 6 experiments. $*P<0.05$,$**P<0.01$ and $***P<0.001$ compared to controls.

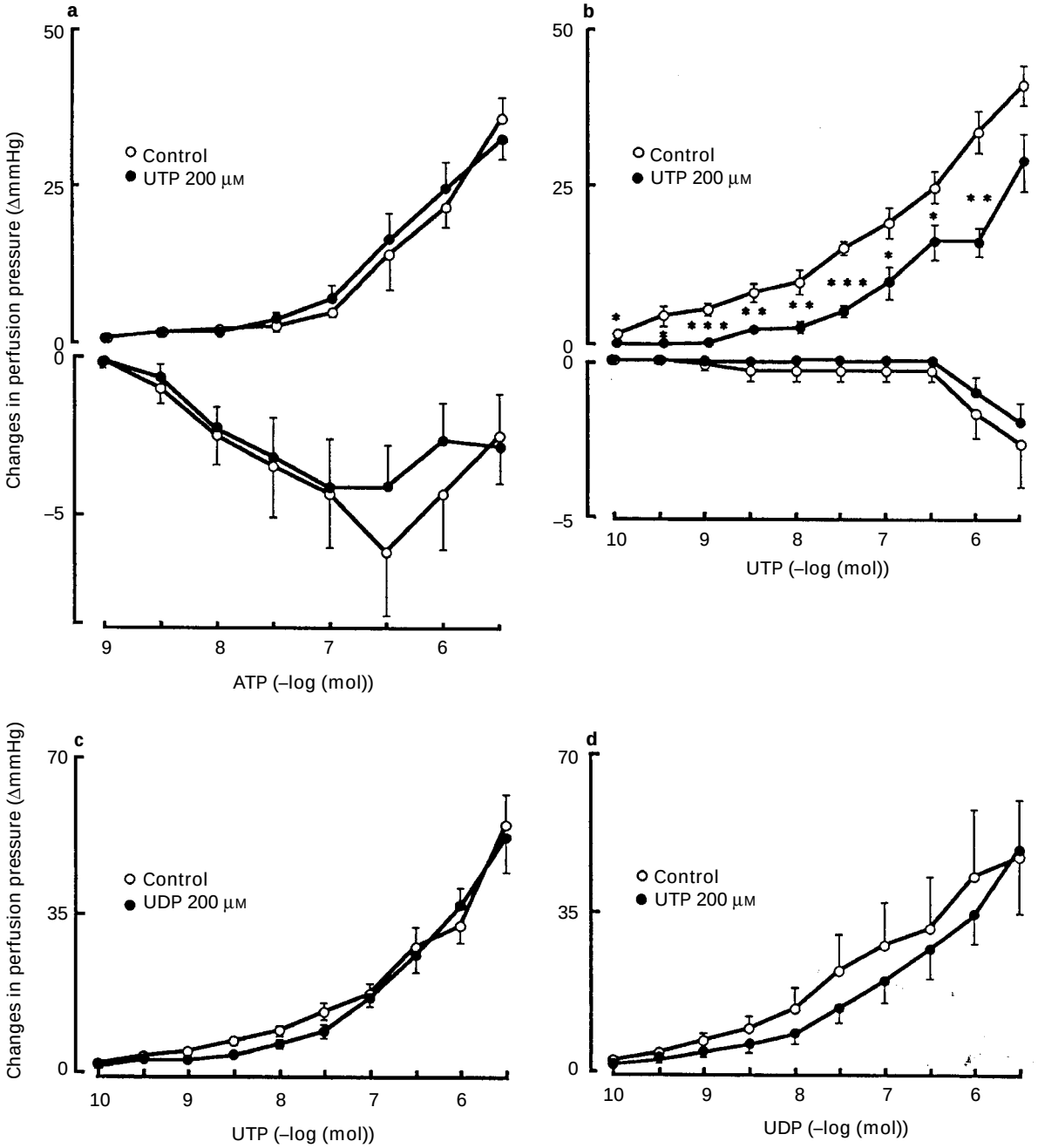

Figure 3 Effects of desensitization with UTP (a,b; 200 μM) and UDP (c,d; 200 μM) on the vascular responses of the canine isolated and perfused epicardial coronary artery to ATP, UTP and UDP. Each point represents the mean and vertical lines show s.e.mean, of 7–8 experiments. $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ compared to controls.

(200 μM) did not affect the vascular responses to ATP and UDP (Figures 3a and d), but it inhibited the vasoconstrictor responses to UTP, though not the vasodilator responses (Figure 3b). Perfusion with UDP (200 μM) did not affect the vasoconstrictor responses to UTP (Figure 3c). The vasodilator responses to UTP and UDP were not affected by perfusion with UDP (200 μM) and UTP (200 μM), respectively (data not shown).

Effects of hexokinase treatment on the vasoconstrictor responses to UDP

To check the conversion of UDP to UTP (Nicholas *et al*., 1996), a stock solution of UDP (30 mM) was preincubated with Krebs-Henseleit solution containing 10 uml^{-1} hexokinase and 22 mM glucose for 1 h. Coronary artery preparations were also preincubated for 30 min and perfused with Krebs-Henseleit solution containing 1 uml^{-1} hexokinase and 22 mM glucose for the construction of the dose-response curves for UDP. The hexokinase treatment did not change the vasoconstrictor responses to UDP (data not shown).

Effects of diltiazem and the removal of extracellular Ca^{2+} on the responses to ATP, UTP and UDP

Perfusion with the Ca channel blocker diltiazem (1 μM) for 20 min reduced the perfusion pressure by about 3 mmHg. Diltiazem (1 μM) abolished the vasoconstrictor responses to KCl (30 μmol, data not shown) and inhibited those to UTP (Figure 4b), although it did not affect those to ATP and UDP (Figure 4a and c).

Incubation with Ca^{2+}-free Krebs-Henseleit solution containing 1 mM EGTA for 15 min reduced the perfusion pressure by about 3 mmHg and inhibited the vascular responses to KCl

(30 μmol; from 37±6 mmHg to 8±2 mmHg, $n=8$), ATP (1 μmol), UTP (1 μmol) and UDP (1 μmol) (Figure 4d, e and f). Incubation for 1 h abolished the vascular responses to KCl, ATP and UTP (data not shown). On the other hand, vasoconstrictor responses to prostaglandin $F_{2\alpha}$ (10 nmol) were reduced from 32±7 mmHg to 8±1 mmHG ($n=3$), but not abolished.

Effects of L-NOARG and indomethacin and endothelium removal by saponin on the responses to UTP

After saponin (1 mg) injection, the perfusion pressure greatly increased. The increased perfusion pressure gradually decreased and reached the previous baseline after about 90 min (data not shown). Removal of the endothelium by saponin

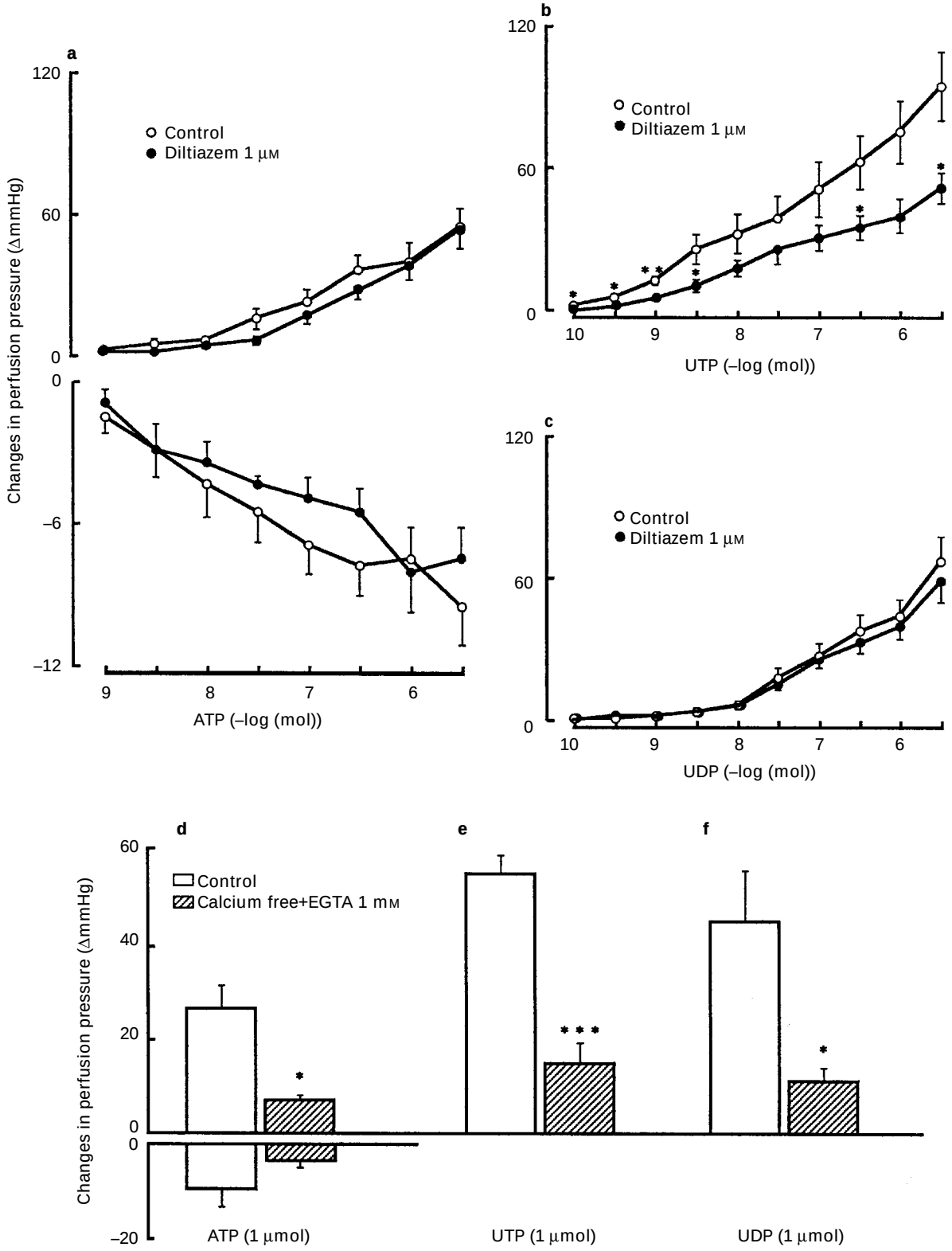

Figure 4 Effects of incubation with diltiazem (1 μM) for 20 min (a–c) and Ca^{2+}-free Krebs-Henseleit solution containing 1 mM EGTA for 15 min; (d–f) on the vascular responses of the canine isolated and perfused epicardial coronary artery to ATP, UTP and UDP. Each point represents the mean, and vertical lines show s.e.mean, of 4–7 experiments. $^{*}P<0.05$ and $^{***}P<0.001$ compared to controls.

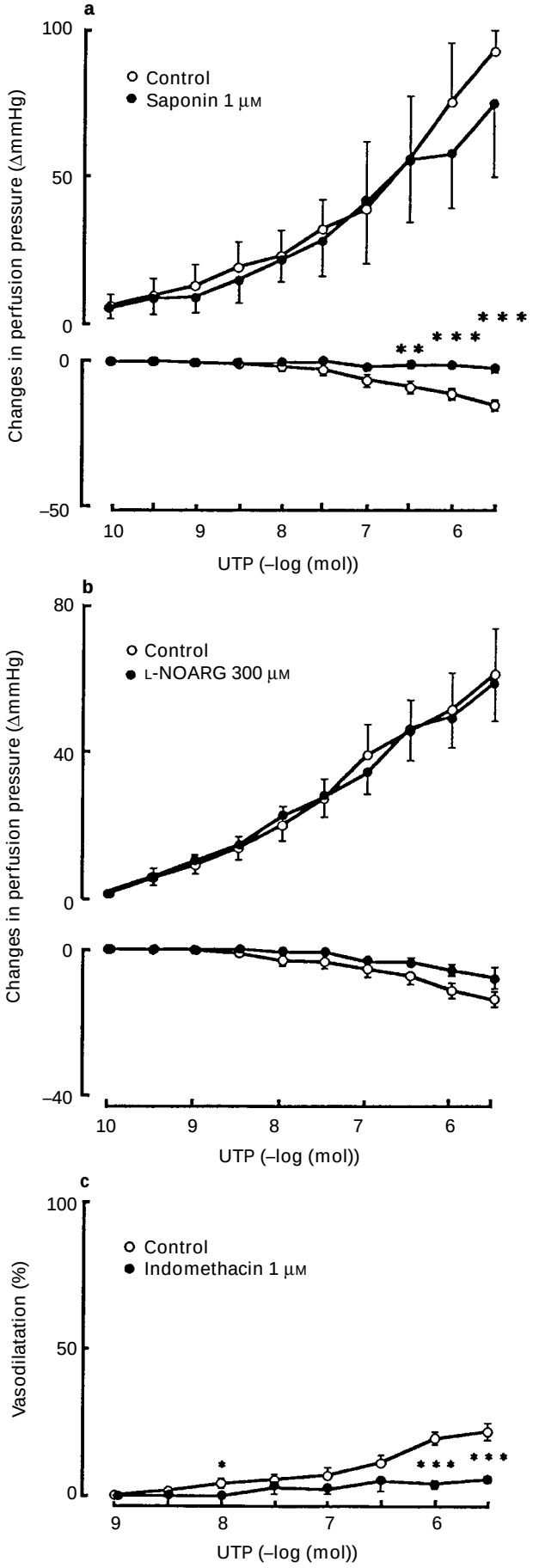

Figure 5 Effects of the removal of endothelium by saponin (a: 1 mg), L-NOARG (b: 300 μM) and indomethacin (c: 1 μM) on the vasodilator responses of the canine isolated and perfused epicardial coronary artery to UTP. Responses were examined after the perfusion pressure had been raised by 20 mM KCl. Each point represents the mean and vertical lines show s.e.mean, of 6–9 experiments. With regard to the effects of indomethacin, vasodilator responses to 1 μmol papaverine were considered to be 100%. $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ compared to controls.

significantly reduced the vasodilator responses to ACh (10 nmol), but it did not affect those to papaverine (1 μmol) as previously shown (Nakane *et al*., 1986). Removal of the endothelium inhibited the vasodilator responses to UTP, but it did not affect the vasoconstriction (Figure 5a).

L-NOARG, an NO synthase inhibitor (300 μM), did not inhibit the vasodilator responses to UTP (Figure 5b). Indomethacin, a cyclo-oxygenase inhibitor (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, the vasodilator responses are presented as the percentage of those to 1 μmol papaverine (Nakane *et al*., 1988). Indomethacin (1 μM) inhibited the vasodilator responses to UTP (Figure 5c).

Discussion

There have been few studies published on the responses of the coronary artery to UTP. UTP induced only vasodilatation or small vasoconstrictor response followed by a large vasodilator response in the Langendorff preparations of the dog (Hashimoto *et al*., 1964) and guinea-pig (Vials & Burnstock, 1993), respectively. In this study, UTP induced a large vasoconstrictor response and only a high dose of UTP sometimes induced a slight vasodilatation following a large vasoconstrictor response. The differences between the results of the past and the present studies may be due to the methods, Langendorff preparation *vs* the perfusion of epicardial coronary artery, namely, the resistance artery vs the epicardial artery. We previously demonstrated that the presence of adrenoceptors varies according to the region of canine epicardial coronary artery studied (Nakane & Chiba, 1986). White and Angus (1987) showed that ATP induced rebound contraction after transient relaxation in the large canine coronary artery, but only relaxation in the small canine coronary artery. UTP may play an important role in coronary vasospasm that affects primarily the large coronary arteries (Maseri *et al*., 1978), because it is a constituent of platelets and may be released from platelets to blood (Goetz *et al*., 1971).

In this study, UTP constricted the canine epicardial coronary artery in a dose-related manner. Vasoconstrictor responses to UTP were not affected by α,β-methylene ATP, suramin and reactive blue 2. α,β-Methylene ATP activates and desensitizes $P2X_1$ and $P2X_3$ receptors (Valera *et al*., 1994; Chen *et al*., 1995). Suramin and reactive blue 2 are non-selective P2 receptor antagonists (Leff *et al*., 1990; Kennedy & Leff, 1995). Thus, P2X receptors do not appear to mediate the vasoconstrictor responses to UTP. Recently, $P2Y_2$, $P2Y_4$ and $P2Y_6$ receptors that mediate the actions of UTP were cloned (Lustig *et al*., 1993; Chang *et al*., 1995; Communi *et al*., 1995; 1996; Nguyen *et al*., 1995). $P2Y_2$ receptors are characterized by the equipotency of UTP and ATP, and low activity of other ATP analogues (Lustig *et al*., 1993). The rank order of agonist potency for vasoconstriction was UTP = UDP > ATP in this study. UTP (200 μM) desensitized the vasoconstrictor responses to UTP, but not those to ATP. Furthermore, diltiazem, a Ca channel blocker (1 μM), inhibited the vasoconstrictor responses to UTP, but not those to ATP. The responses to UTP acting a $P2Y_2$ receptors were suramin-sensitive, but those at $P2Y_4$ receptors were not (Charlton *et al*., 1996). Thus, $P2Y_2$ receptors do not mediate the vasoconstrictor response to UTP. The agonist potency order for elevating intracellular Ca^{2+} in $P2Y_4$ receptors (Communi *et al*., 1995) is the same as that, UTP = UDP > ATP, for vasocon-

striction in this study. However, UTP and UDP did not shown cross-desensitization. Nicholas *et al*. (1996) showed that $P2Y_6$ receptors have selective affinity with UDP and that hexokinase treatment reduced the responses to UDP mediating $P2Y_2$ and $P2Y_4$ receptors. Hexokinase treatment did not reduce the vasoconstrictor responses to UDP in this study. α,β-Methylene ATP and suramin also did not inhibit those to UDP. Thus, $P2Y_4$ and $P2Y_6$ receptors may be involved in the vasoconstrictor responses to UTP and UDP in the canine epicardial coronary artery, respectively.

In this study, the Ca channel blocker diltiazem (1 μM) inhibited the vasoconstrictor responses to UTP, but not those to ATP and UDP. Removal of extracellular Ca^{2+} inhibited those to the nucleotides. This suggests that the receptors activated by UTP but not those by ATP and UDP are coupled to L-type Ca ion channels, although the responses to the nucleotides are dependent on extracellular Ca^{2+}. In the rat tail and femoral arteries and the canine saphenous vein, the Ca channel blocker nicardipine and incubation with Ca^{2+}-free solution inhibited the vasoconstrictor responses to UTP (Saïag *et al*., 1990). In the bovine middle cerebral arterial strips, the increases of intracellular Ca^{2+} and the contractile forces induced by UTP were reduced after incubation with Ca^{2+}-free solution containing 2 mM EGTA (Miyagi *et al*., 1996). The rise in intracellular Ca^{2+} induced by UTP was independent of extracellular Ca^{2+} in the rat aortic smooth muscle cells (Kitajima *et al*., 1994; Pacaud *et al*., 1995) and the human cultured coronary artery smooth muscle cells (Strøboek *et al*., 1996). The cultured smooth muscle cells were incubated with Ca^{2+}-free solution for only 2–5 min. Phenotypes may be changed during culture (Pacaud *et al*., 1995). The cloned P2Y receptors that have high affinity with UTP and UDP are G-protein-coupled receptors and increase intracellular inositol 1,4,5-triphosphate and Ca^{2+} (Nicholas *et al*., 1996). However, there is no study that checked the dependency of the extracellular Ca^{2+} in the cloned P2Y receptors. P2X receptors are involved in the vasoconstrictor responses to ATP in the canine epicardial coronary artery (Matsumoto *et al*., 1997). P2X receptors are ligand-gated ion channels (Brake *et al*., 1994; Valera *et al*., 1994). α,β-Methylene ATP and suramin did not inhibit the vasoconstrictor responses to UDP, suggesting that P2X receptors are not involved in the vasoconstrictor responses to UDP. Therefore, the UTP-preferring P2Y receptors are coupled to L-type Ca ion channels, but UDP-preferring P2Y receptors are not coupled to channels in the canine epicardial coronary artery. Further studies are needed to understand the relationships between activation of P2Y receptors by UTP and UDP and Ca handling.

The removal of endothelium by saponin (Nakane *et al*., 1986) inhibited the vasodilator responses to UTP, but it did not affect the vasoconstriction. L-NOARG (300 μM), an NO synthase inhibitor (Moore *et al*., 1990), like N^{ω}-nitro-L-arginine methyl ester, did not affect the vasodilator responses to UTP. L-NOARG (300 μM) inhibits ATP-induced vasodilatation in the canine epicardial coronary artery (Matsumoto *et al*., 1997). Thus, the release of NO is not involved in the vasodilator responses of the canine epicardial coronary artery to UTP. Indomethacin (1 μM), a cyclo-oxygenase inhibitor, inhibited UTP-induced vasodilatation in this study. UTP induced the release of prostacyclin from cultured endothelial cells of the pig aorta (Needham *et al*., 1987), the bovine aorta (Kitazono *et al*., 1992; Wilkinson *et al*., 1993), the bovine pulmonary artery (Lustig *et al*., 1992) and the guinea-pig coronary artery (Yang *et al*., 1996). Therefore, UTP probably dilates the canine epicardial artery mainly through the release of prostacyclin from the endothelium.

UTP induced a small vasodilator response (less than 15 mmHg at raised perfusion pressure) at high doses in the canine epicardial coronary artery. The rank order of potency for vasodilatation was $ATP>>ITP \geqslant UDP>UTP \geqslant TTP$, which does not correspond to that proposed for P2Y receptors. The non-selective P2 receptor antagonist reactive blue 2 (Kennedy & Leff, 1995) did not inhibit the vasodilator responses to UTP, but did inhibit those to ATP in the canine epicardial coronary artery (Matsumoto *et al*., 1997). UTP (200 μM) did not desensitize the vasodilator responses to UTP, although it reduced the vasoconstrictor responses to UTP in this study. Indomethacin (1 μM) inhibited the vasodilator responses to UTP (this study) but not those to ATP (Matsumoto *et al*., 1997) in the canine epicardial coronary artery. L-NOARG (300 μM) did not inhibit the vasodilator responses to UTP (this study), but did inhibit those to ATP (Matsumoto *et al*., 1997). These results suggest that the receptors mediating the vasodilatation induced by UTP are different from those mediating the vasoconstriction induced by UTP and mediating the vasodilatation by ATP.

In conclusion, we analysed the responses of the canine epicardial coronary artery to UTP. UTP induces vasoconstriction via UTP-preferring P2Y receptors on the smooth muslce and vasodilatation via receptors, different from those mediating vasoconstrictor response to UTP and vasodilator responses to ATP, on the endothelium mainly through the release of prostacyclin in the canine epicardial coronary artery. UDP induces vasoconstriction via UDP-preferring P2Y receptors. L-type Ca ion channels are involved in the vasoconstriction induced by UTP, but not in that induced by UDP. Further studies are needed to understand the relationships between activation of P2Y receptors by UTP and UDP and Ca handling.

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