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Nucleotide-evoked relaxation of human coronary artery

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Abstract

Endothelium-dependent dilation of coronary blood vessels in response to ATP and related nucleotides has been demonstrated in various animal species. The aim of the present study was to investigate a possible relaxant effect of ATP, the adenine nucleotides 2-methylthio ATP (MeSATP) and adenosine 5′-*O*-(2-thiodiphosphate) (ADPβS), and the pyrimidine nucleotide UTP in isolated human coronary artery. In endothelium-intact rings of human coronary artery precontracted with K^+ (20–40 mM), the nucleotides caused relaxation. Average maximal percentage relaxations and average EC_{50} values (concentrations causing half-maximal relaxation) were 89% and 47.1 μM for ATP, 28% and 0.3 μM for MeSATP, 35% and 0.6 μM for ADPβS, and 49% and 1.6 μM for UTP. For each of the four agonists, the potency to elicit relaxation varied greatly between individual rings, so that equi-relaxing concentrations spanned several orders of magnitude. Moreover, the sensitivities to ATP and UTP, when tested in the same ring, were not correlated. Mechanical removal of the endothelium as well as N^G-nitro-L-arginine methyl ester (L-NAME; 30 μM), an inhibitor of nitric oxide synthase, abolished the relaxation caused by MeSATP, ADPβS and UTP and greatly attenuated the response to lower concentrations of ATP (3.2–320 μM), but high concentrations of ATP (320 and 1000 μM) caused relaxation also in endothelium-denuded preparations and in the presence of L-NAME. High concentrations of ADPβS (32 and 100 μM) and UTP (320 and 1000 μM) caused contraction of endothelium-denuded preparations. Thus, extracellular nucleotides cause endothelium-dependent, primarily nitric oxide-mediated relaxation of human coronary artery. ATP in addition causes endothelium-independent relaxation. The receptors activated by the nucleotides appear to be unevenly distributed on the coronary endothelium.

Keywords: Coronary artery; Human; Endothelium; Vasodilation; P2 receptor; Nucleotide; UTP

1. Introduction

In the cardiac vasculature of several animal species, the adenine nucleotides ATP, ADP, 2-methylthio ATP (MeSATP) and adenosine 5′-*O*-(2-thiodiphosphate) (ADPβS) cause vasodilation (Fleetwood and Gordon, 1987; Hardebo et al., 1987a; Hopwood and Burnstock, 1987; Houston et al., 1987; White and Angus, 1987; Kelm and Schrader, 1990; Lee et al., 1990; Mombouli et al., 1991; Brown et al., 1992; Keef et al., 1992; Corr and Burnstock, 1994; Vials and Burnstock, 1994a,b; Simonsen et al., 1997). In addition, endogenous ATP has been suggested to contribute to the autoregulation of coronary blood flow (Hopwood et al., 1989; see Burnstock, 1989 for review). The relaxation-mediating receptors activated by ATP and related nucleotides are located on the endothelium and couple to the liberation of prostacyclin or nitric oxide in guinea pig and dog heart (Houston et al., 1987; White and Angus, 1987; Kelm and Schrader, 1990; Lee et al., 1990; Mombouli et al., 1991; Brown et al., 1992; Vials and Burnstock, 1994b). In rabbit heart, in contrast, the relaxation-mediating receptors are located directly on the smooth muscle cells (Keef et al., 1992; Corr and Burnstock, 1994). The pyrimidine nucleotide UTP likewise causes endothelium-dependent vasodilation in the guinea pig coronary vasculature (Vials and Burnstock, 1993) and receptors for UTP have been demonstrated in cultured vascular endothelial cells from rabbit and guinea pig heart (Mannix et al., 1993; Yang et al., 1996).

In man, intravenous infusion of ATP increases coronary blood flow, and ATP is used as a tool in the diagnosis of coronary artery disease (e.g., Miyagawa et al., 1995). Yet, in contrast to the well-funded knowledge in laboratory animals, nothing is known about the effects of extracellular

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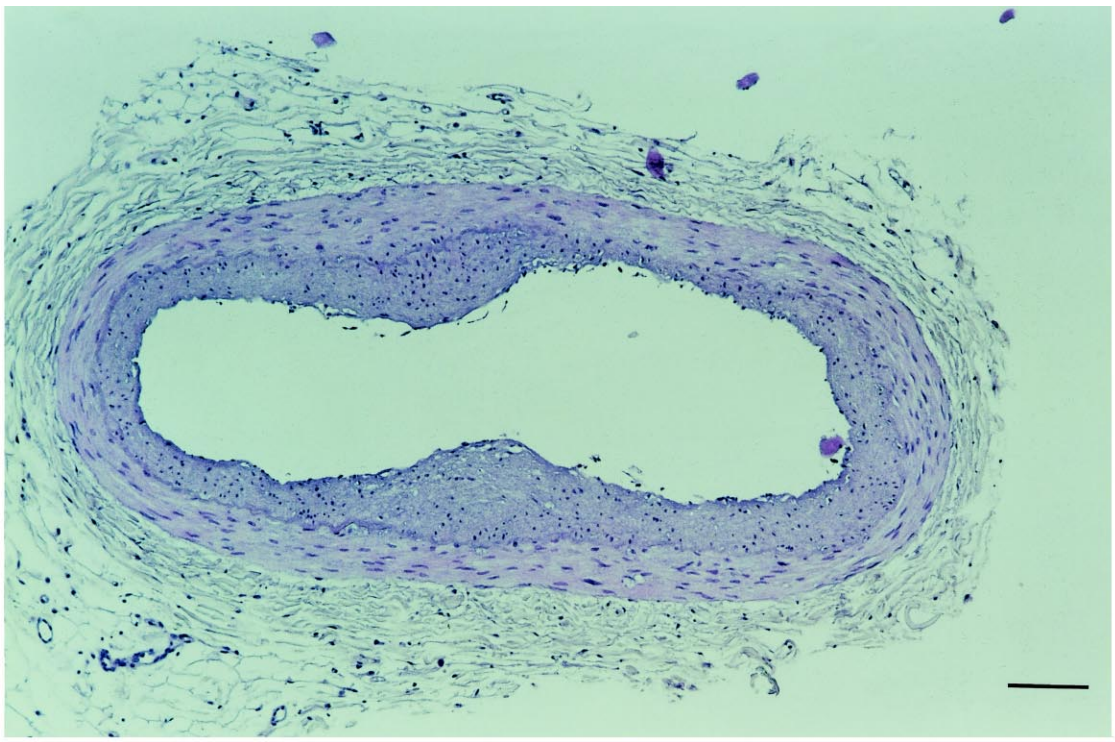

Fig. 1

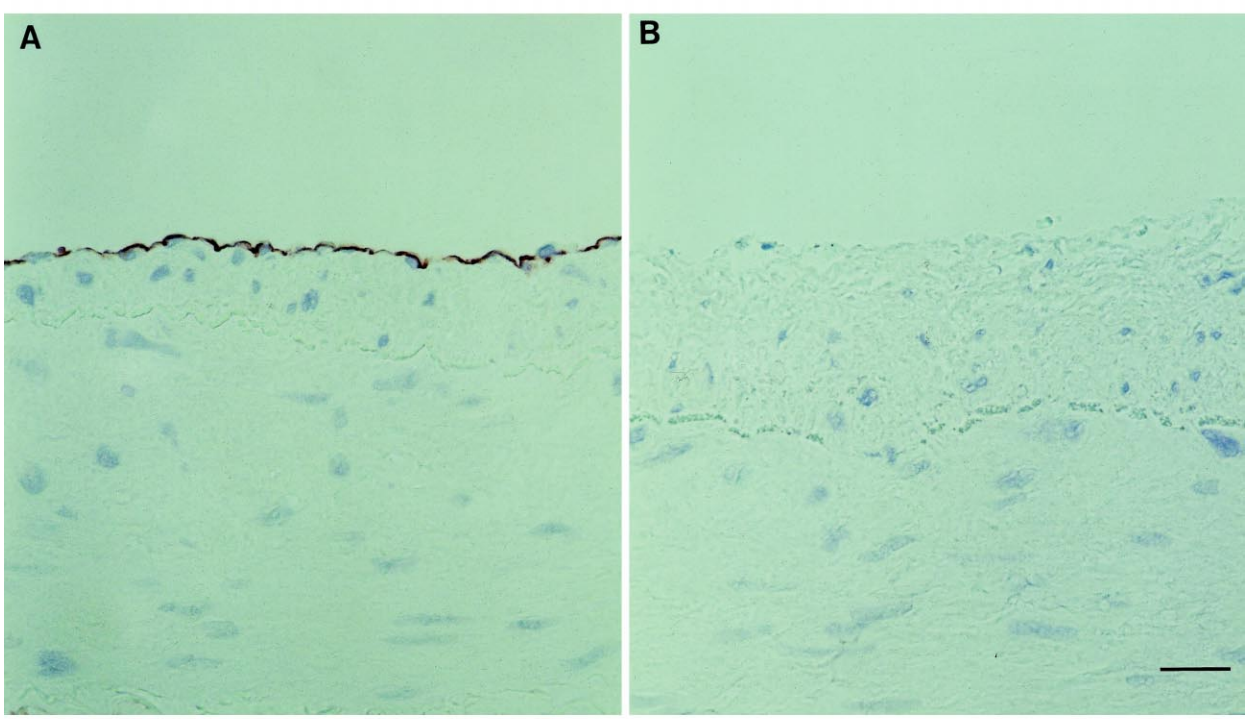

Fig. 2

Fig. 1. Cross-section of a human coronary artery ring. Artery from a patient with dilated cardiomyopathy (III, ring no. 4 in Table 1). Low-power magnification (1:100) of hematoxylin–eosine staining. Note concentric intimal fibrosis with superimposed cushion-like thickening and some foam cells due to atherosclerosis. Scale bar = 100 μm.

Fig. 2. Cross-sections of human coronary artery rings. Artery from a patient with dilated cardiomyopathy (IV, (A) ring no. 1 in Table 1; in (B), the endothelium was mechanically removed). High-power magnifications (1:400) of CD 34 immunohistochemistry with positive brown staining of the endothelium in A and lack of staining in B. Scale bar = 25 μm.

nucleotides in isolated human coronary artery. The aim of the present study, therefore, was to investigate a possible relaxant effect of ATP, MeSATP, ADPβS and UTP in K^+-precontracted human coronary artery. MeSATP, ADPβS and UTP were used because they act at pharmacologically distinct receptors for extracellular nucleotides (see Fredholm et al., 1997).

2. Materials and methods

2.1. Preparation

Human coronary arteries were obtained from two donor hearts that could not be transplanted for technical reasons, and from five end-stage failing hearts obtained during transplantation; four hearts were from patients with dilated cardiomyopathy and one heart was from a patient suffering from congenital heart disease. The age of the donors and patients was 43 ± 6 years (median: 36, range: 26–61 years); three were women and four were men. In vitro experiments using human cardiac tissue had been approved by the Ethical Committee of the University Clinics of Freiburg.

A piece of the left ventricular free wall of the hearts was excised and stored, during transport to the laboratory (20 min–6 h), at 4°C in a cardioplegic Tyrode's solution containing 2,3-butanedione monoxime (30 mM; Mulieri et al., 1989) and saturated with 95% O_2/5% CO_2. The left anterior descending coronary artery was removed, cleaned of adherent tissue and cut into rings of about 4 mm in length. In four rings, the endothelium was removed by gently rubbing the intimal surface.

2.2. Tension measurement

The rings were mounted in a 5.9-ml organ bath. Two stainless steel hooks were inserted through the lumen; the lower hook was fixed and the upper one was attached to an isometric force transducer (K30, Hugo Sachs Elektronik, Hugstetten, Germany) coupled to a thermal pen recorder (Graphtec, Ettlingen, Germany). The incubation medium contained: 118 mM NaCl, 4.8 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 0.9 mM KH_2PO_4, 25 mM $NaHCO_3$, 11 mM glucose, 0.3 mM ascorbic acid and 0.03 mM disodium EDTA. It was saturated with 95% O_2/5% CO_2 and kept at 37°C. Unless stated otherwise, it was replaced every 20 min.

During a 60-min equilibration period, the resting tension was twice adjusted to 9.8 mN. Increasing concentrations of KCl were then added in a cumulative fashion (beginning with 20 mM; increments of 5 mM) in order to determine, in each individual ring, the lowest concentration of K^+ to produce a stable contraction. The reason was

Table 1
Preservation of endothelium, relaxation caused by histamine, and relaxant potency of ATP and UTP in individual endothelium-intact rings of human coronary artery

Heart (diagnosis)[a]	Ring no.	Percentage of intact endothelium	Percentage of relaxation caused by histamine (1 μM)	Concentration (μM) causing 30% relaxation	
				ATP	UTP
I (DCM)	3	92	70	667	contraction only
	6	95	86	5.1	10.0
II (CHD)	1	54	54	81.3	< 10% relaxation
III (DCM)	1	57	70	96.0	11.5
	4	89	95	12.6	0.8
	5	22	25	3.5	12.6
IV (DCM)	1	77	49	3.2	0.5
	2	85	86	5.2	1.3
	8	50	32	109	5.6
	10	94	70	1.9	8.6
V (donor)	2	68	91	147	20.5
	4	49	67	11.8	contraction only
	5	49	65	23.4	contraction only
	6	67	51	35.4	contraction only
VI (donor)	4	47	68	90.9	0.2
	5	72	74	103	0.6

The table contains all rings in which both ATP and UTP were tested. Concentrations causing 30% relaxation were interpolated from the nearest data points of concentration–response curves in Fig. 4.
[a] Dilated cardiomyopathy (DCM); congenital heart disease (CHD).

that endothelium-dependent relaxation is more pronounced when the concentration of K^+ used for contraction is low (cf. Sabouni et al., 1990b). The concentration of KCl thus determined (20–40 mM) was then administered three times—100, 120 and 180 min after the start of the experiment (first to third precontraction).

The first precontraction served to examine the condition of the endothelium. Histamine (1 μM) was added during the plateau of the K^+ response. The endothelium was considered intact when histamine caused at least 25% relaxation (as throughout this paper, relaxation was quantified as a percentage of the respective K^+ precontraction); it was considered removed when histamine failed to elicit relaxation (cf. Toda and Okamura, 1989; Stork and Cocks, 1994). Rings that did not satisfy these criteria were discarded.

The second and third precontractions served to determine concentration-response curves for nucleotide-induced relaxation. Nucleotides were administered in a cumulative fashion during the plateau of the K^+ response, i.e., from 5 to 20 min after the addition of KCl onwards. They were washed out together with KCl when the relaxation elicited by the highest concentration was maximal. It took 10 to 30 min to determine one concentration–relaxation curve. When, at high nucleotide concentrations, transient or persistent contractions developed, the stable tension level immediately before addition of the next higher concentration was evaluated. Two different nucleotides were tested in each preparation, the first one during the second and the second one during the third precontraction.

In some experiments, the first nucleotide was added again during a fourth precontraction, 240 min after the start of the experiment.

2.3. Histochemistry

At the end of the experiment, the coronary artery rings were fixed in 4% unbuffered formalin. Serial cross-sections were stained with hematoxylin and eosin or used for immunohistochemistry. The primary antibody was directed against the CD 34 protein (QBEND 10; 1:250; Immunotech, Hamburg, Germany) which is present on en-

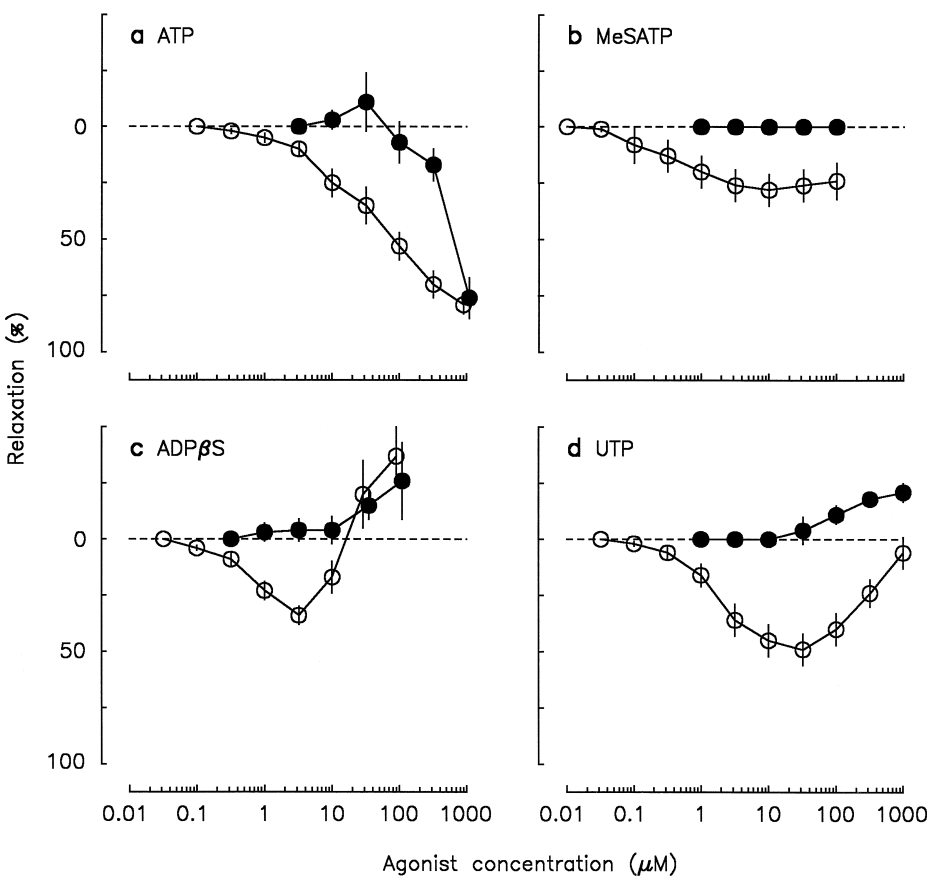

Fig. 3. Response of K^+-precontracted rings of human coronary artery to nucleotides: averaged concentration-response curves. Tissues were precontracted by KCl (20–40 mM) twice; interval: 60 min. Increasing concentrations of ATP (a), 2-methylthio ATP (MeSATP) (b), adenosine 5′-*O*-(2-thiodiphosphate) (ADPβS) (c) or UTP (d) were administered in a cumulative fashion during the plateau of either the first or the second response to K^+. Abscissae, agonist concentration. Ordinates show relaxation in preparations with intact endothelium (○), and preparations from which the endothelium had been removed (●), as a percentage of the respective response to K^+. Means ± S.E.M. from 3–23 experiments. Note that, in the case of UTP, those rings are included in which the nucleotide caused no relaxation, but only contraction (see Fig. 4d).

dothelial cells. Immunostaining was performed using a modified three-step avidin–biotin complex method (Hsu et al., 1981; Schaefer, 1984). Peroxidase activity was visualized by 3-amino-9-ethylcarbazole to yield a brown reaction product. Sections were slightly counterstained with hematoxylin. One cross-section from each ring was morphometrically evaluated to determine the percentage of the luminal border covered with endothelial cells (CD 34-positive). Control experiments were performed by staining samples of the internal mammary artery (obtained during bypass surgery; with a well-known CD 34 reactivity seen in the endothelium), and by substituting the primary antibody with nonimmune mouse serum. The positive controls were invariably positive and the negative controls were invariably negative.

2.4. Statistics

Data are expressed as the arithmetic mean ± S.E.M. For the computation of maximal effects and EC_{50} values of agonists (concentrations producing 50% of the respective maximum), logistic curves were fitted to weighted mean percentage relaxation values by means of Eq. 25 of Waud (1976) and nonlinear regression. When a concentration-response relationship was bell-shaped (Fig. 3b,c,d), the responses to concentrations higher than the maximally relaxant one were taken to be identical with the maximal relaxation. The EC_{50} and maximal effect are given with their S.E. as defined by Waud (1976).

2.5. Materials

2-Methylthio ATP tetrasodium (MeSATP; Biotrend, Köln, Germany), adenosine 5′-*O*-(2-thiodiphosphate) trilithium (ADPβS), ATP disodium, histamine hydrochloride, N^G-nitro-L-arginine methyl ester (L-NAME) and UTP trisodium (Sigma, Deisenhofen, Germany) were dissolved in distilled water. KCl was dissolved in medium. Solutions of drugs were added to the organ bath in aliquots not exceeding 100 μl.

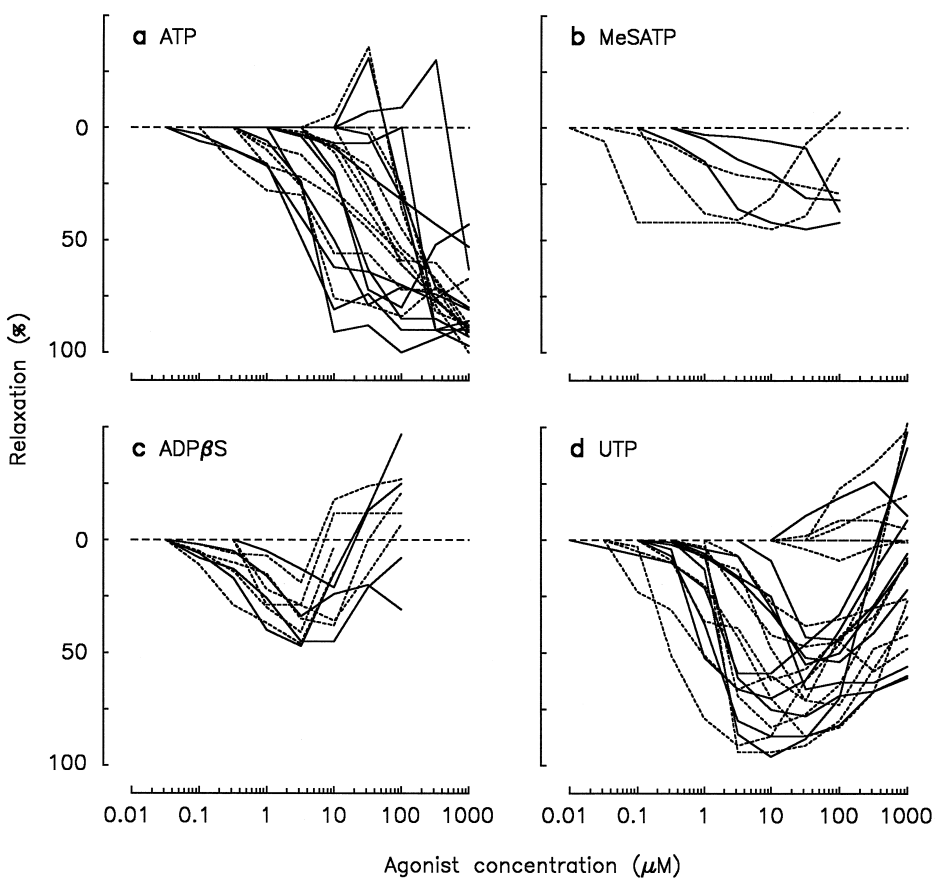

Fig. 4. Response of K^+-precontracted rings of human coronary artery to nucleotides: individual experiments in preparations with intact endothelium. Tissues were precontracted by KCl (20–40 mM) twice; interval: 60 min. Increasing concentrations of ATP (a), 2-methylthio ATP (MeSATP) (b), adenosine 5′-*O*-(2-thiodiphosphate) (ADPβS) (c) or UTP (d) were administered in a cumulative fashion during the plateau of either the first or the second response to K^+. Abscissae, agonist concentration. Ordinates show relaxation as a percentage of the respective response to K^+. Each line represents an individual concentration–relaxation curve. Solid lines indicate rings in which histamine (1 μM) elicited at least 70% relaxation.

3. Results

3.1. General

All coronary arteries displayed at least diffuse concentric intimal fibrosis. In addition, focal cushion-like intimal hyperplasia with typical foam cells as a sign of atherosclerosis was present in 57% of the coronary rings (Fig. 1), including those from the two donor hearts.

In the 30 rings of this study with intact endothelium (> 25% relaxation of precontracted rings in response to 1 μM histamine), 66 ± 4% of the luminal border was covered with intact endothelium (CD 34-positive; Fig. 2A; no significant difference between rings from donor and recipient hearts; $P > 0.05$). The percentage relaxation produced by histamine (1 μM; 62 ± 4% on average) was positively, though weakly, correlated with the percentage of intact endothelium ($r = 0.39$; $n = 30$; $P < 0.05$; see also Table 1). In the four coronary rings in which the endothelium had been removed, the percentage of the luminal circumference covered by CD 34-positive cells was reduced to 6 ± 4% (Fig. 2B) and histamine (1 μM) caused no relaxation, but further contraction.

3.2. Nucleotide-evoked responses

In endothelium-intact rings, the contraction elicited by K^+ (20–40 mM) averaged 9.0 ± 0.6 mN ($n = 30$; first precontraction). When added in a cumulative fashion during the plateau of the contraction elicited by K^+, low concentrations of ATP, MeSATP, ADPβS and UTP caused increasing relaxation (open symbols in Fig. 3). At higher concentrations, transient or, in the case of ADPβS and UTP (Fig. 3c,d), persistent contractions developed.

The EC_{50} values (and maximal percentage relaxations) obtained by sigmoid curve fitting were 47.1 ± 10.9 μM (89 ± 2%) for ATP ($n = 21$), 0.33 ± 0.07 μM (28 ± 1%) for MeSATP ($n = 6$), 0.60 ± 0.20 μM (35 ± 4%) for ADPβS ($n = 10$) and 1.6 ± 0.17 μM (49 ± 1%) for UTP ($n = 23$).

For each of the four nucleotides, the potency to elicit relaxation varied greatly, so that equi-relaxing concentrations spanned several orders of magnitude in various coronary rings (Fig. 4). UTP, which in several rings caused prominent relaxation at concentrations below 10 μM, caused little relaxation, or even contraction only, in five other rings (Fig. 4d). Even those rings in which histamine (1 μM) produced prominent relaxation (≥ 70%) displayed variable sensitivity to all four nucleotides (uninterrupted lines in Fig. 4). On the other hand, relaxations in one and the same ring were reproducible: a second concentration–relaxation curve of ATP, ADPβS or UTP in the same ring (during a fourth precontraction by K^+; see Section 2) was close to the first one (during the second precontraction; $n = 2–3$; not shown).

In several rings, both ATP and UTP were tested. The results of these experiments are summarized in Table 1. As expected from Fig. 4, the concentration causing 30% relaxation varied greatly for either nucleotide, between 1.9 and 667 μM for ATP and between 0.2 and 20.5 μM for UTP. The sensitivities to ATP and UTP were not correlated: in rings I/6, III/5 and IV/2, ATP and UTP were approximately equipotent at causing relaxation (less than four-fold

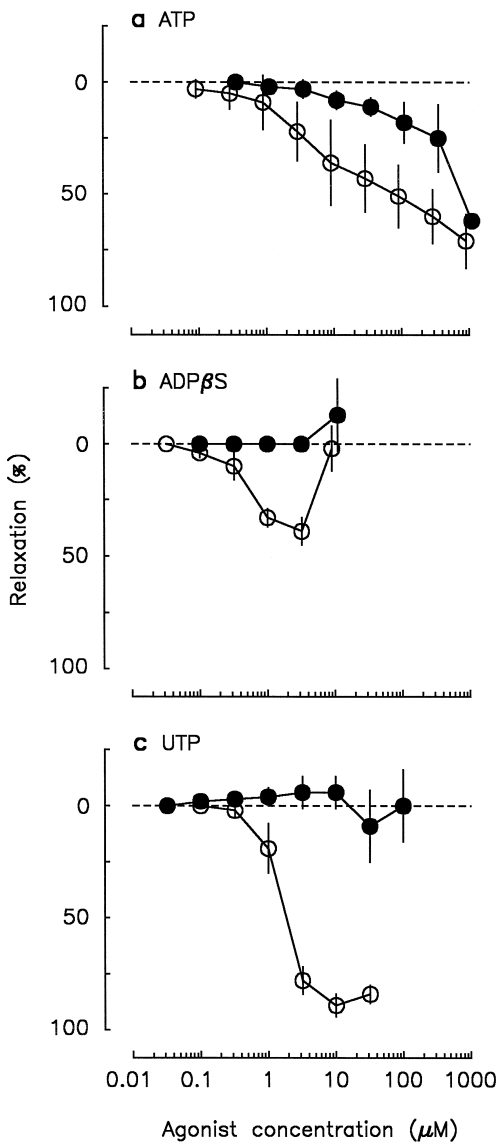

Fig. 5. Response of K^+-precontracted rings of human coronary artery to nucleotides: effect of N^G-nitro-L-arginine methyl ester (L-NAME). Tissues were precontracted by KCl (20–40 mM) three times; interval: 60 min. Increasing concentrations of ATP (a), adenosine 5′-*O*-(2-thiodiphosphate) (ADPβS) (b) or UTP (c) were administered in a cumulative fashion during the plateau of the first and third response to K^+. L-NAME (30 μM) was added 20 min before the third addition of K^+, i.e., about 30 min before the second ATP, ADPβS or UTP concentration–relaxation curve. Abscissae, agonist concentration. Ordinates show relaxation in first curves (○), and second curves in the presence of L-NAME (●), as a percentage of the respective response to K^+. Means ± S.E.M. from three experiments each.

difference); in rings III/4, IV/8, VI/4 and VI/5, UTP was at least 10 times more potent than ATP; and in rings II/1, V/4, V/5 and V/6, ATP was moderately potent, while UTP caused less than 10% relaxation or only contraction. The relative sensitivity to ATP and UTP differed also in rings from the same heart (III, IV and V in Table 1).

The nitric oxide synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME; 30 μM), when added during a fourth precontraction by K^+ (see Section 2), did not alter the resting tension of the rings or the plateau of the precontraction elicited by K^+. It abolished the relaxation produced by ADPβS and UTP (Fig. 5b,c) and greatly attenuated the response to lower concentrations of ATP (3.2–320 μM; Fig. 5a).

In endothelium-denuded rings, the contraction elicited by K^+ (20–40 mM) averaged 10.1 ± 2.0 mN ($n = 4$; first precontraction; not significantly different from endothelium-intact rings). MeSATP, ADPβS and UTP caused no relaxation, and ADPβS and UTP instead elicited further contraction (filled symbols in Fig. 3b,c,d). High concentrations of ATP, on the other hand, produced a slowly developing relaxation also in endothelium-denuded preparations (filled symbols in Fig. 3a). Note that the concentration–relaxation curve of ATP in endothelium-denuded preparations is similar to the curve in the presence of L-NAME (Fig. 5a).

4. Discussion

4.1. General

All tissue samples used in the present study displayed diffuse intimal fibrosis, about half of them in addition mild atherosclerosis (see Fig. 1). The preparations used, thus, represent a relatively homogeneous population in terms of the degree of visible morphologic lesions. None of the rings displayed signs of severe atherosclerosis.

However, there was a marked variability between individual rings in the percentage of the luminal border covered with intact endothelium (see Table 1). The relaxation caused by histamine (1 μM) was positively correlated with the percentage of intact endothelium and was abolished by removal of the endothelium. It may, therefore, be taken as a measure for the functional integrity of the endothelium.

4.2. Nucleotide-evoked responses

ATP, MeSATP, ADPβS and UTP relaxed K^+-precontracted rings of human coronary artery. Removal of the endothelium abolished the response to MeSATP, ADPβS and UTP (Fig. 3b,c,d), as did L-NAME in the case of ADPβS and UTP (Fig. 5b,c). The relaxation caused by these nucleotides, therefore, was entirely due to activation of receptors located on the endothelium, and nitric oxide was the primary mediator released. This also holds true for the effect of low concentrations of ATP (up to 32 μM; Fig. 3a and Fig. 5a). Endothelium-dependent, nitric oxide-mediated coronary vasodilation caused by ATP, MeSATP, ADPβS and UTP has also been demonstrated in guinea pigs and dogs (see Section 1). The present results, of course, do not exclude the possibility that prostacyclin and endothelium-derived hyperpolarizing factor (EDHF) contribute to the action of the nucleotides in human coronary arteries under physiological conditions.

The potency of the nucleotides to elicit endothelium-dependent relaxation varied considerably between single preparations—even between rings taken from the same heart (Fig. 4; Table 1). Two nucleotides were tested in each preparation (see Section 2); desensitization of the tissue following addition of the first nucleotide, therefore, might be a reason for the observed variability. This possibility is made unlikely, however, by the fact that a second concentration–response curve to ATP, ADPβS and UTP in the same ring was close to the first. Marked differences were observed even in those rings in which histamine (1 μM) elicited prominent relaxation (Fig. 4; Table 1), indicating that the variability was neither due to a general endothelial dysfunction in some rings, but not in others, nor to a different degree of precontraction by K^+. The differing potencies may rather reflect variations in the distribution of the nucleotide receptors on the coronary endothelium.

Our results demonstrate for the first time endothelium-dependent relaxation of human coronary artery in response to ATP, MeSATP, ADPβS and UTP. With respect to the type of endothelial receptors, they permit the following tentative considerations.

Adenosine has been shown to cause relaxation of human coronary arteries by activation of smooth muscle A_2 receptors (Ramagopal et al., 1988; Sabouni et al., 1990a,b; Makujina et al., 1992). It seems unlikely that these receptors contribute to the endothelium-dependent, relaxant effect of MeSATP, ADPβS, UTP and low concentrations of ATP. Higher concentrations of ATP, in contrast, might—after degradation to adenosine—cause endothelium-independent relaxation of human coronary artery through activation of smooth muscle A_2 receptors.

Of the nucleotide P2 receptors presently known (see Fredholm et al., 1997), ADPβS and MeSATP preferentially activate the $P2Y_1$ subtype, which is thought to represent the 'classical' P_{2Y} purinoceptor (Burnstock and Kennedy, 1985). Relaxation-mediating P_{2Y} purinoceptors have been demonstrated in the cardiac vasculature of several species (Fleetwood and Gordon, 1987; Hopwood and Burnstock, 1987; Houston et al., 1987; Keef et al., 1992; Corr and Burnstock, 1994; Vials and Burnstock, 1994b), and it seems likely that this subtype is also the site of action of ADPβS and MeSATP in the human coronary artery. In support of this notion, a human $P2Y_1$ receptor

has recently been cloned from vascular endothelial cells (Ayyanathan et al., 1996; Léon et al., 1996).

UTP is not an agonist at the $P2Y_1$ receptor (Fredholm et al., 1997). The pyrimidine nucleotide might act either at a $P2Y_2$ receptor, i.e., the 'classical' P_{2U} purinoceptor (activated by both UTP and ATP; O'Connor et al., 1991), or at a $P2Y_4$ or $P2Y_6$ receptor, i.e., a 'pyrimidinoceptor' (activated by UTP, but insensitive to ATP; Häussinger et al., 1987; Von Kügelgen et al., 1987). Both P_{2U} purinoceptors and 'pyrimidinoceptors' have been demonstrated in cultured vascular endothelial cells from rabbit or guinea pig heart (Mannix et al., 1993; Yang et al., 1996). In the present study, the order of potency of ATP and UTP to elicit relaxation varied considerably between single rings of human coronary artery (Table 1). Some rings even responded to one of the two nucleotides, but not to the other. These findings argue against a common receptor for ATP and UTP, and therefore in favor of an action of UTP, at least in part, at a 'pyrimidinoceptor'.

ATP, finally, may activate both endothelial P_{2Y} and P_{2U} purinoceptors (Pirotton et al., 1993). The present results give no information about its site of action in human coronary artery. The varying order of potency of ATP and UTP argues against an exclusive action of ATP at a common receptor for the two nucleotides, i.e, a P_{2U} subtype.

At concentrations higher than needed for relaxation, the nucleotides caused transient or persistent contraction of endothelium-intact rings. Contraction was the only response to ADPβS and UTP in endothelium-denuded rings (Fig. 3). The contraction, therefore, presumably was mediated by receptors located on the smooth muscle cells. It may represent a correlate of the nucleotide-evoked intracellular Ca^{2+} transients recently demonstrated in isolated human coronary artery smooth muscle cells and thought to be mediated by both P_{2Y} and P_{2U} purinoceptors (Strøbæk et al., 1996).

Vasoconstrictor and vasodilator responses to ATP, UTP and related nucleotides have been demonstrated in human pial arteries (Urquilla, 1978; Hardebo et al., 1987b), pulmonary arteries (Greenberg et al., 1987; Liu et al., 1989), subcutaneous and omental resistance arteries (Martin et al., 1991) and in the circulation of the placenta (Read et al., 1993; Dobronyi et al., 1997). In addition, vasoconstriction in response to extracellular nucleotides has been shown in human mesenteric vein and skeletal arteries (Pernow et al., 1987) and saphenous vein (Von Kügelgen et al., 1995). The present findings extend this list and support the idea that extracellular nucleotides contribute to the regulation of vascular tone also in man.

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