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In vitro studies of release of adenine nucleotides and adenosine from rat vascular endothelium in response to α_1-adrenoceptor stimulation

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1 Noradrenaline-induced release of endogenous adenine nucleotides (ATP, ADP, AMP) and adenosine from both rat caudal artery and thoracic aorta was characterized, using high-performance liquid chromatography with fluorescence detection.

2 Noradrenaline, in a concentration-dependent manner, increased the overflow of ATP and its metabolites from the caudal artery. The noradrenaline-induced release of adenine nucleotides and adenosine from the caudal artery was abolished by bunazosin, an α_1-adrenoceptor antagonist, but not by idazoxan, an α_2-adrenoceptor antagonist. Clonidine, an α_2-adrenoceptor agonist, contracted caudal artery smooth muscle but did not induce release of adenine nucleotides or adenosine.

3 Noradrenaline also significantly increased the overflow of ATP and its metabolites from the thoracic aorta in the rat; however, the amount of adenine nucleotides and adenosine released from the aorta was considerably less than that released from the caudal artery.

4 Noradrenaline significantly increased the overflow of ATP and its metabolites from cultured endothelial cells from the thoracic aorta and caudal artery. The amount released from the cultured endothelial cells from the aorta was also much less than that from cultured endothelial cells from the caudal artery. In cultured smooth muscle cells from the caudal artery, a significant release of ATP or its metabolites was not observed.

5 These results suggest that there are vascular endothelial cells that are able to release ATP by an α_1-adrenoceptor-mediated mechanism, but that these cells are not homogeneously distributed in the vasculature.

Keywords: ATP; rat thoracic aorta; rat caudal artery; endothelial cells; ATP release; α_1-adrenoceptor; noradrenaline

Introduction

Adenosine triphosphate (ATP), in addition to its known functions in intracellular metabolism, is increasingly being recognized for its extracellular functions in various tissues, including blood vessels (Gordon, 1986; White, 1988; Burnstock, 1991). Recently, it has been suggested that one endogenous source of extracellular ATP is vascular endothelium (Pearson & Gordon, 1979; Buxton *et al.*, 1990; Milner *et al.*, 1990; Sedaa *et al.*, 1990). We also observed a large release of ATP and its metabolites induced by methoxamine, an α_1-adrenoceptor agonist from the caudal artery of rats (Shinozuka *et al.*, 1991); however, details of the release have not been revealed.

In the present study, we measured the release of endogenous adenine nucleotides (ATP, ADP, AMP) and adenosine from both rat caudal artery and thoracic aorta to characterize the release of ATP.

Methods

Tissue preparations

Male Wistar rats (SLC, Japan), ranging from 20 to 25 weeks of age, were anaesthetized with pentobarbital sodium (50 mg kg^{-1} i.p.) and exsanguinated. The caudal arteries and thoracic aortas were removed, immersed in a continuously oxygenated Krebs–Henseleit buffer solution and cleaned of connective tissue. The composition of the Krebs solution was as follows (mM): NaCl 118.4, KCl 4.7, $CaCl_2$ 2.2, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25.0 and glucose 5.6. In the release experiments, the caudal arteries (approximately 11 cm in length and 10 mg wet weight) were either cut open lengthwise (open preparation) or tied at both ends (closed preparation). For the measurement of contractile activity, the caudal artery was cut into small rings 2–3 mm in length.

Release experiments

The caudal artery and thoracic aorta preparations were suspended in water-jacketed organ chambers containing 2.0 ml of the Krebs solution at 37°C, then allowed to equilibrate for 1 h. The medium was replaced every 3 min during the second half of the equilibration period. After a 60 min equilibration period, the bathing solution was rapidly collected by draining the organ chamber every 3 min. After the first samples were taken (basal overflow), the tissues were stimulated with noradrenaline (1.0 μM) for 3 min, and the bathing solutions (noradrenaline-induced overflow) were collected. The details of the experimental protocol have been described previously (Ishii *et al.*, 1993).

To obtain data for the concentration-release curve in response to noradrenaline, caudal arteries were removed from Wistar rats. Each artery was divided into four segments, which were then opened lengthwise. After the first samples were taken, each of the four open-preparation segments was stimulated with noradrenaline at four concentrations (0.1, 1.0, 10.0 and 100.0 μM), for 3 min each in a random sequence.

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Contraction experiments

The ring preparations of the caudal artery were suspended under a resting tension of 0.5 g in a water-jacketed micro-organ chamber containing 2.0 ml of the Krebs solution at 37°C and then allowed to equilibrate for 1 h. The tension was measured isometrically with a force-displacement transducer (Iwashiya Kishimoto Medical Instruments, Japan) and recorded with a Hitachi Recorder (Model 561, Japan). The details of the method for measuring the tension changes have been described previously (Shinozuka *et al.*, 1992). A concentration–contraction curve for noradrenaline was generated in a cumulative manner, and each successive concentration was added after the response to the previous concentration had reached its peak.

To compare the contractions induced by noradrenaline and by clonidine, caudal arteries from four rats were stimulated, first with noradrenaline and then with clonidine, at 30 min intervals. Another four arteries were stimulated first with clonidine and then with noradrenaline. The values were expressed as a percentage of the maximum contraction induced in each artery by a 3 min treatment with 1.0 μM noradrenaline.

Cell cultures

Endothelial and smooth muscle cells in primary cultures from either the thoracic aorta or caudal artery of the rat were prepared by methods previously described (Ohoka *et al.*, 1990; Hashimoto *et al.*, 1992). Briefly, after excising caudal arteries and thoracic aortas from the 5–6 rats anaesthetized with ether, ranging from 20 to 25 weeks of age, the vessels were rinsed in Dulbecco's modified Eagle medium (DMEM) containing antibiotics (100 U ml^{-1} penicillin, 100 μg ml^{-1} streptomycin) and an antifungal agent (0.25 μg ml^{-1} fungizone).

To obtain endothelial cells for the primary culture, vessels were cut into flat segments of approximately 2 mm^2. Each segment was placed endothelial side down on a well of a collagen culture plate (Celltight C-1, Sumitomo Bakelite, Osaka, Japan), to which just enough growth medium [50 μg ml^{-1} ECGF, endothelial cell growth factor and 3% fetal bovine serum (FBS) in Opti-MEM®1] was added to keep the tissue moist. The segments were incubated in a humidified incubator at 37°C in a 95% air and 5% CO_2 atmosphere. Within 48 h, the segments were removed from the culture plates, and the growth medium was exchanged for a renewal medium (25 μg ml^{-1} ECGF, 3% FBS and 5U ml^{-1} heparin in DMEM). The remaining cells were cultivated for a further 3–5 days, during which time the renewal medium was changed every 2–3 days, and the cells were subjected to release experiments of adenine nucleotides and adenosine after growing to confluent monolayers.

To obtain smooth muscle cells for the primary culture of rat caudal arteries, the artery was opened longitudinally, and the adventitia and intima were carefully removed from the media. The media was cut into segments of 1–2 mm^2 and explanted to dishes containing DMEM with 10% FBS. The dishes were incubated for approximately 2 weeks in a humidified incubator at 37°C in a 95% air and 5% CO_2 atmosphere. After cultivation for 2 weeks, the cells grew into clumps like those observed in smooth muscle cells cultured from guinea-pig vas deferens (Chamley *et al.*, 1974). This suggests that the cultured smooth muscle cells in the present experiments are predominantly of the contractile phenotype, which is characteristic of the cells in normal physiological conditions.

To identify endothelial cells and smooth muscle cells in primary culture, immunocytochemical characterization was performed using monoclonal antibodies against factor VIII-related antigen (a Biomeda Histo Scan Kit, COSMO BIO, Tokyo, Japan) and smooth muscle α-actin (Sigma, St Louis, MO, U.S.A.) and also using acetylated low-density lipoprotein labelled with the fluorescent probe 1,1′-dioctadecyl-1-3,3,3′,3′-tetramethyl-indocarbocyanine perchlorate (Dil-Ac-LDL; Biomedical Technologies, Stoughton, MA, U.S.A.), as previously described (Hashimoto *et al.*, 1992). Endothelial cells showed positive staining for factor VIII-related antigen but undetectable staining for α-actin and also showed uptake of Dil-Ac-LDL, while smooth muscle cells were negative for factor VIII-related antigen and positive for α-actin (data not shown).

In the release experiments, the cells in the primary cultures were incubated on culture dishes in 2 ml of Krebs solution for 60 min at 37°C in an atmosphere of 95% air and 5% CO_2 ($20-25 \times 10^4$ cells per 35 mm dish for endothelial cells, $25-30 \times 10^4$ cells per 35 mm dish for smooth muscle cells). After 60 min of equilibration, 200 μl of the bathing solution was collected (basal overflow), after which cells were stimulated with noradrenaline (10.0 μM) for 3 min, followed by another 200 μl collection (stimulated overflow). The collected samples were then processed for the measurement of adenine nucleotides and adenosine as described above.

Measurement of ATP and its metabolites

The collected sample solutions were immediately acidified to pH 4 with 0.36 ml of citrate–phosphate buffer solution and placed on ice. Chloroacetaldehyde and internal standard (9-β-D-arabinofuranosyl adenine) were added to each acidified sample, which was then incubated in a dry bath at 80°C for 40 min. The reaction was terminated by placing the samples on ice. The resulting ethenonucleotides and ethenoadenosine were analysed by high-performance liquid chromatography (HPLC) with fluorescence detection as described by Mohri *et al.*, (1993).

The HPLC system consisted of a reversed-phase Ultron-N-phenyl HPLC column (Shinowa-Kakoh, Japan), a delivery pump (LC-9A, Shimadzu, Japan) equipped with a fluorescence monitor (RF-535, Shimadzu, Japan), a sample processor (AS-8010, Tosoh, Japan) and a chromatopac computing integrator (C-R4A, Shimadzu, Japan).

Drugs

Clonidine hydrochloride, α,β-methylene ADP, idazoxan hydrochloride and [−] noradrenaline bitartrate were obtained from Sigma, U.S.A. Bunazosin was kindly supplied by Eisai Pharmaceutical, Japan. The DMEM was obtained from Nissui Pharmaceutical, (Japan). The ECGF was purchased from Boehringer Mannheim (Mannheim, Germany). Opti-MEM1 and FBS were purchased from Gibco Laboratories (U.S.A.).

Statistics

All data were expressed as means ± standard error of the mean. The significance of difference between the amounts of purine overflow before and after treatment with noradrenaline was analysed by paired *t*-test. The other data were evaluated for statistical significance by using Student's *t*-test, the Cochran–Cox test or Duncan's multiple range test where appropriate. The 0.05 level of probability was accepted as significant.

Results

In the rat caudal artery, noradrenaline, at a concentration of 1 μM, significantly increased the overflow of ATP, ADP, AMP and adenosine from the caudal artery segments which were opened lengthwise (Figure 1). Adenosine (4.41 ± 0.72 pmol mg^{-1}, $n = 4$) was present in the greatest amount, which was approximately equal to the sum of those of the nucleotides: ATP, ADP and AMP (3.74 ± 0.37 pmol mg^{-1}, $n = 4$). α,β-methylene ADP, a nucleotidase inhibitor, at

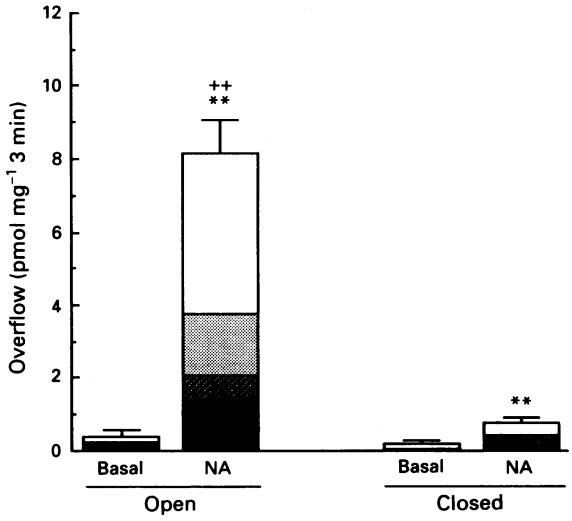

Figure 1 Effect of 1.0 μM noradrenaline (NA) on the overflow of adenine nucleotides [ATP (solid column), ADP (diagonal hatched column), AMP (dotted column)] and adenosine (ADO, open columns) from rat caudal artery. Noradrenaline significantly (**$P<0.01$, paired t-test) increased the total overflow of adenine nucleotides and adenosine in both preparations. The total amount of adenine nucleotides and adenosine overflow induced by noradrenaline from open preparations was significantly larger (++$P<0.01$) than that from closed preparations. Each column represents the mean of four experiments. The vertical bar on each column indicates standard error of the mean of the total amount of purines released.

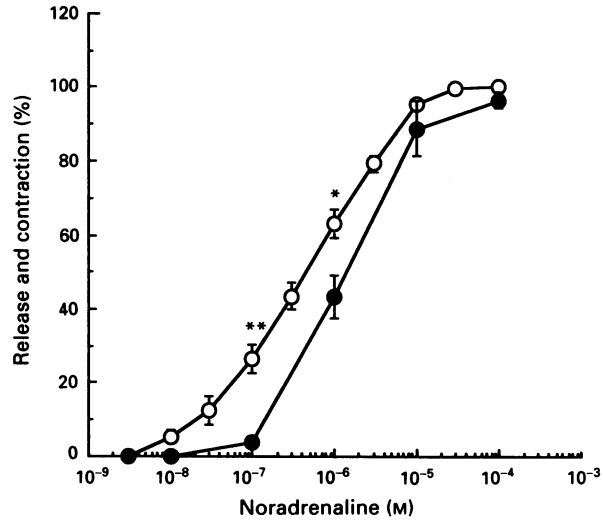

Figure 2 Noradrenaline-induced release of adenine nucleotides and adenosine and noradrenaline-induced contraction curves for rat caudal artery. The release curve (●) represents the relationship between the total amount of adenine nucleotides and adenosine released (per cent of the mean of the maximal release) and the concentration of noradrenaline used to induce release from open caudal artery preparations. The amount released is the difference between the amounts present in basal and in noradrenaline-induced overflow. The second curve (○) is the per cent contraction induced by specified concentrations of noradrenaline in ring preparations of the rat caudal artery. Each point represents the mean of six experiments. The single and double asterisks indicate significant differences (*$P<0.05$ and **$P<0.01$ respectively) between the per cent release and per cent contraction.

0.1 μM significantly ($P<0.05$, $n=4$) increased the noradrenaline-induced overflow of ATP from 1.606 ± 0.120 to 3.007 ± 0.124 pmol mg^{-1}. In intact arteries that had been tied closed at both ends, noradrenaline (1.0 μM) also increased the overflow of adenine nucleotides and adenosine. However, the total amount of adenine nucleotides and adenosine released, which was the difference between the overflow in the absence (basal) and presence of noradrenaline, from closed preparations (0.57 ± 0.09 pmol mg^{-1}) was significantly less ($P<0.01$) than that from open preparations (7.76 ± 0.86 pmol mg^{-1}).

The noradrenaline-induced release of adenine nucleotides and adenosine was concentration-dependent from 0.1 to 100.0 μM. Noradrenaline at a concentration of 100.0 μM produced maximal release of adenine nucleotides and adenosine, which amounted to 22.02 ± 2.20 pmol mg^{-1}, and at 0.01 μM produced no release. The concentration–response curve for purine release induced by noradrenaline was shifted slightly to the right in comparison with the concentration–response curve for contraction induced by noradrenaline in the caudal artery (Figure 2). However, the EC_{50} values for release and contraction were 1.81 ± 0.73 μM ($n=6$) and 0.49 ± 0.10 μM ($n=6$) respectively, and there was no significant difference between these two EC_{50} values.

The increase in adenine nucleotides and adenosine overflow in response to 1 μM noradrenaline (control, 9.90 ± 2.14 pmol mg^{-1}, $n=5$) was significantly ($P<0.05$) reduced to 0.81 ± 0.31 pmol mg^{-1} ($n=4$) by 30.0 nM bunazosin, an α_1-adrenoceptor antagonist, but was not affected by 0.1 μM idazoxan (9.39 ± 1.31 pmol mg^{-1}, $n=4$), an α_2-adrenoceptor antagonist. Clonidine, an α_2-adrenoceptor agonist, produced a contraction of the caudal artery when administered at a final concentration of 10 μM, the magnitude and time course of which was almost identical to that produced by 1.0 μM noradrenaline (Figure 3a). However, neither 1.0 nor 10.0 μM clonidine caused any significant increase in purine overflow (Figure 3b).

In rat thoracic aorta, noradrenaline also increased the overflow of adenine nucleotides and adenosine (Figure 4a). However, the total amount of adenine nucleotides and adenosine released (noradrenaline-induced overflow minus basal overflow) from the thoracic aorta (0.22 ± 0.04 pmol mg^{-1}) was significantly less ($P<0.01$) than that obtained from the caudal artery (7.91 ± 0.77 pmol mg^{-1}). When the amount of release was compared on the basis of area (mm^2) instead of weight (mg) of tissue, the release of purines from aorta (0.06 ± 0.02 pmol mm^{-2}, $n=4$) was still significantly ($P<0.01$) less than that obtained from the caudal artery (1.59 ± 0.14 pmol mm^{-2}, $n=4$).

In cultured endothelial cells that had been obtained from either the caudal artery or the thoracic aorta, noradrenaline, at a concentration of 10.0 μM, significantly increased the overflow of total purines (Figure 4b). There was no significant difference between the basal overflow levels of adenine nucleotides and adenosine from cultured endothelial cells from the aorta and caudal artery. However, the total amount of noradrenaline-induced release of adenine nucleotides and adenosine from endothelial cells of the caudal artery (3.45 ± 1.12 pmol per 10,000 cells) was significantly greater ($P<0.05$) than that from the aorta (0.21 ± 0.06 pmol per 10,000 cells), with that of the former approximately 16 times that of the latter. The amount of nucleotides (3.41 ± 1.14 pmol per 10,000 cells, $n=6$) released from primary cultures of endothelial cells from the caudal artery by 10 μM noradrenaline was significantly greater ($P<0.05$) than that of adenosine (0.03 ± 0.02 pmol per 10,000 cells, $n=5$), as shown in Figure 4b. In a primary culture of rat caudal artery smooth muscle cells, we did not detect a significant release of purines induced by 10 μM noradrenaline (Figure 4b).

Discussion

In the present study, we have shown that noradrenaline induced the release of ATP, ADP, AMP and adenosine from the caudal artery segments that had been opened lengthwise.

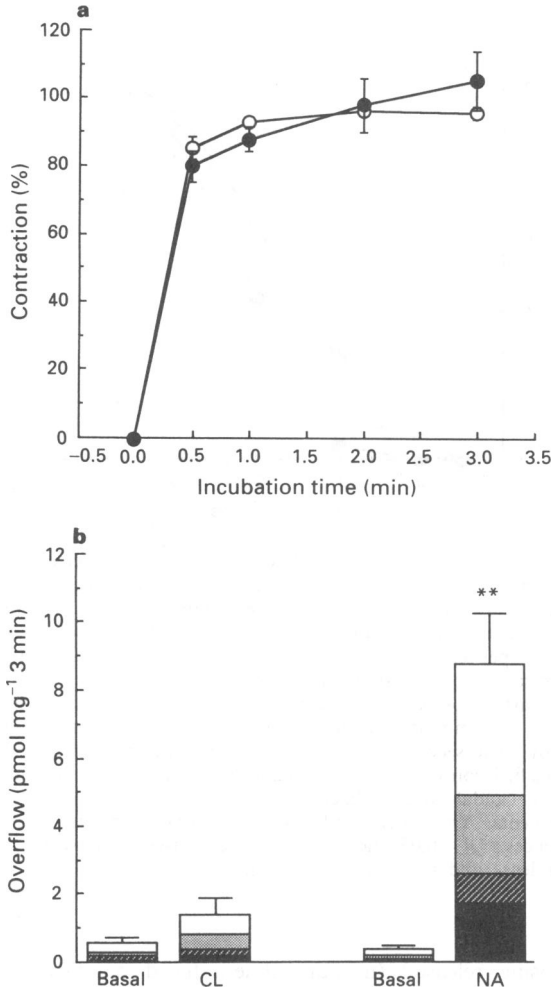

Figure 3 Comparison of the effects of noradrenaline (NA) and clonidine (CL) on both contraction and purine overflow in rat caudal artery. (a) Magnitude and time course of the contractile responses induced by noradrenaline (○, 1.0 μM) and clonidine (●, 10.0 μM) for 3 min with ring preparations of the rat caudal artery. The values are expressed as the per cent of the maximal contraction induced by a 3-min treatment of noradrenaline in each artery. Each point represents the mean of eight experiments. (b) Comparison of the effects of 10.0 μM clonidine and 1.0 μM noradrenaline on the overflow of adenine nucleotides and adenosine from open preparations of rat caudal artery. Noradrenaline significantly ($*P<0.05$, paired t-test) increased the total overflow of adenine nucleotides and adenosine. Each column represents the mean of four experiments. Vertical bars on each column indicate standard error of the mean of the total amounts of adenine nucleotides and adenosine present in each experimental group. Columns as in Figure 1.

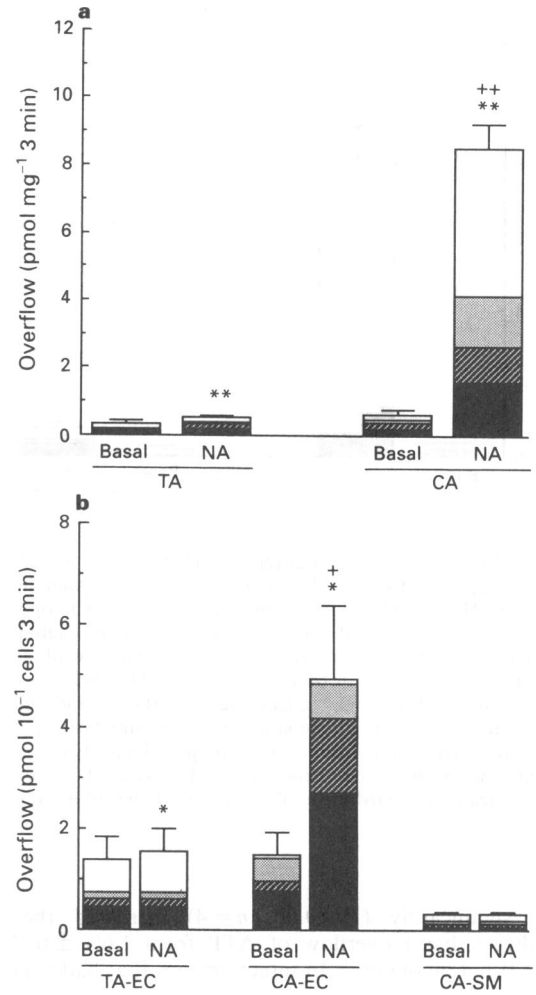

Figure 4 Comparison of the effects of noradrenaline (NA) on the overflow of adenine nucleotides and adenosine from the thoracic aorta and caudal artery of the rat. (a) Effects of 1.0 μM noradrenaline on the overflow of adenine nucleotides and adenosine from open preparations of the thoracic aorta (TA) and caudal artery (CA). Noradrenaline significantly ($**P<0.01$, paired t-test) increased the total overflow of adenine nucleotides and adenosine in both tissues. The total amount of adenine nucleotides and adenosine overflow induced by noradrenaline from the caudal artery was significantly greater ($^{++}P<0.01$) than that obtained from the thoracic aorta. Each column represents the mean of five experiments. Vertical bars on each column indicate standard error of the mean of the total amounts of adenine nucleotides and adenosine present in each experimental group. (b) Effect of 10.0 μM noradrenaline on the overflow of adenine nucleotides and adenosine from isolated endothelial cells in primary cultures which were obtained from the thoracic aorta (TA-EC) and caudal artery (CA-EC) and smooth muscle cells in primary cultures which were obtained from the caudal artery (CA-SM). Noradrenaline significantly ($*P<0.05$, paired t-test) increased the total overflow of adenine nucleotides and adenosine from the endothelial cells. The total amount of noradrenaline-induced overflow of adenine nucleotides and adenosine from endothelial cells of the caudal artery was much greater ($^{+}P<0.05$) than that from the aorta. Data were obtained from 5–6 rats. Each column represents the mean of the values averaged in two or three observations in different dishes of cells per rat. Vertical bars on each column indicate standard error of the mean of the total amount of adenine nucleotides and adenosine present in each group. Columns as in Figure 1.

In intact arteries that had been tied closed at both ends, noradrenaline also induced the release of adenine nucleotides and adenosine, but the total amount of adenine nucleotides and adenosine released by closed arteries was only 7% of that released by open arteries. This indicates that most (approximately 93%) of the adenine nucleotides and adenosine released by noradrenaline in the open arteries comes from the intimal side of the artery, most probably from the endothelium. Indeed, we observed that the release of purines was considerably reduced in a rat caudal artery that had been denuded of endothelium by rubbing the lumen of the vessel (data not shown). This is consistent with our previous results (Sedaa *et al.*, 1990) with rabbit thoracic aorta, in which the release of purines by methoxamine, an α_1-adrenoceptor agonist, was reduced by 90% by removal of the endothelium.

Very recently, work in our laboratory has shown that a variety of vasoactive agents, including bradykinin, acetylcholine, serotonin and ADP, cause purine release from primary cultures of endothelial cells obtained from guinea pig heart (Yang *et al.*, 1994). This suggests that agonist-induced release of adenine nucleotides and adenosine may not be

restricted to α_1-receptor agonists. It is possible that noradrenaline activates α_2- or β_2-adrenoceptors in additon to α_1-adrenoceptors. However, the noradrenaline-induced release of purines was almost abolished by bunazosin, an α_1-adrenoceptor antagonist (Shoji, 1981), but was not affected by idazoxan, an α_2-adrenoceptor antagonist. Clonidine, an α_2-adrenoceptor agonist, produced a contraction of the caudal artery, the magnitude and time course of which were almost identical to that produced by noradrenaline. However, clonidine did not cause any significant increase in purine overflow. Also, isoproterenol (1.0 μM), a β-adrenoceptor agonist, did not affect the overflow of purines (data not shown). From these results, the α-adrenoceptor that mediates purine release appears to be the α_1-receptor, a finding that is consistent with our previously reported observations (Buxton *et al.*, 1990; Sedaa *et al.*, 1990).

It is well known that the release of EDRF, endothelium-derived relaxing factor, from endothelial cells can be elicited by mechanical and physical stimulation, such as flow shear stress (Rubanyi *et al.*, 1986) and stretch (Ohno *et al.*, 1990). Furthermore, Milner *et al.*, (1990) demonstrated that endothelin and ATP release can be induced from rabbit aortic endothelial cells by shear stress. In the present study, the concentration–response curve for purine release was similar to the concentration–response curve for contraction of the caudal artery, and the EC_{50} values for release and contraction were not significantly different. This suggests the possibility that mechanical contraction may induce the release of adenine nucleotides and adenosine. However, the release of ATP that was observed in the present study seems not to be induced by mechanical stimulation, such as contraction, because clonidine produced a contraction but did not induce release. These findings taken together suggest that adenine nucleotides and adenosine are released by an α_1-adrenoceptor-coupled mechanism that is present in endothelial cells.

In rat thoracic aorta, noradrenaline also increased the overflow of adenine nucleotides and adenosine, but the amount of release was only approximately 3% of that obtained from the caudal artery, despite there being no significant difference between the basal total purine overflow levels from the aorta and caudal artery. When the amount of release was compared on the basis of area instead of weight of tissue, as described previously (Shinozuka *et al.*, 1993), the release of adenine nucleotides and adenosine from aorta was still significantly less than that from the caudal artery. Thus, there appears to be a marked difference between the amount of purines released from the aorta and that from the caudal artery.

In cultured endothelial cells from either the caudal artery or the thoracic aorta, noradrenaline induced the release of total purines. There was no significant difference between the basal overflow levels of adenine nucleotides and adenosine from cultured endothelial cells from the aorta and those from the caudal artery. However, the noradrenaline-induced release of adenine nucleotides and adenosine from cultured endothelial cells from the aorta was again much less than that from cells from the caudal artery, with the former approximately 6% of the latter. This release of purines from specific endothelial cell populations, therefore, agrees with the results from intact caudal artery and thoracic aorta. Thus, these experimental results suggest that a functional role exists for an α_1-adrenoceptor-mediated release of purines on the endothelial cells which is regionally differentiated in the vasculature.

The amount of nucleotides released by noradrenaline from primary cultures of endothelial cells from the caudal artery was significantly greater than that of adenosine. However, in intact caudal artery preparations, the amount of adenosine released was greater than that of nucleotides. Pearson & Gordon (1979) also showed that the quantity of adenine nucleotides released from cultured endothelial cells was greater than that of adenosine, and Gordon *et al.* (1986) suggested that the degradation of ATP to adenosine by endothelial cell ectonucleotidases is not very rapid. In contrast, these investigators showed that ATP is rapidly degraded to adenosine by cultured smooth muscle cells from pig aorta (Gordon *et al.*, 1989). Indeed, we observed that the amount of ATP released by noradrenaline from the opened caudal artery of rat was increased approximately 2-fold by the 5′-nucleotidase inhibitor, α,β-methylene ADP. Thus, most of the ATP released from endothelial cells by α_1-adrenoceptor stimulation may be rapidly hydrolysed to adenosine by ectonucleotidase on the surface of smooth muscle cells in the caudal artery of the rat.

In a primary culture of rat caudal artery smooth muscle cells, we did not detect a significant release of adenine nucleotides or adenosine induced by noradrenaline. Bodin *et al.*, (1991) also reported that shear stress did not evoke ATP release in cultured vascular smooth muscle cells. These results suggest that vascular smooth muscle cells do not possess a significant ATP-releasing function, but are important in the metabolic conversion of ATP to adenosine.

Extracellular ATP and its metabolites are known to produce a number of biological and pharmacological effects in blood vessels by acting on specific cell-surface purinoceptors on adrenergic nerves (Shinozuka *et al.*, 1988; 1991; Yokoyani *et al.*, 1992; Ishii *et al.*, 1993), smooth muscles (Burnstock, 1991) and endothelium (Burnstock, 1987; Ando *et al.*, 1991; Vials & Burnstock, 1993). Although it is not possible to calculate from our present data the exact concentration of endogenous ATP released from the endothelium that is then available to interact with tissue-specific purinoceptors, a reasonable approximation of the amount of purines per tissue volume can be made and is in the neighbourhood of 20 μM. Based on literature values, this concentration of ATP is in the range to effectively interact with purinoceptors of vascular tissues. From both the present results and these approximations, it is proposed that ATP and its metabolites that are released from the endothelium have important physiological or pathophysiological functions in the vasculature.

In conclusion, the present results strongly suggest that (1) an α_1-adrenoceptor-mediated mechanism for ATP release exists on the vascular endothelial cells and (2) functional heterogeneity involving this α_1-adrenoceptor-mediated release of ATP exists in the vasculature. A heterogeneous anatomical distribution of α_1-adrenoceptors on vascular endothelial cells may well explain the differences in the noradrenaline-induced ATP release in the rat caudal artery and thoracic aorta. In addition, the functions of ATP and its metabolites may be more important in the lower resistance blood vessels rather than in larger vessels, such as the aorta.

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