Regional Differences in Noradrenaline-Induced Release of Adenosine Triphosphate from Rat Vascular Endothelium

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Abstract

We examined the release of endogenous adenylyl purines such as adenosine triphosphate (ATP), ADP, AMP and adenosine from the caudal artery (CA), saphenous artery (SA), renal artery (RA), mesenteric artery (MA), pulmonary artery (PA) and thoracic aorta (TA) of rats, using high-performance liquid chromatography fluorescence detection. Noradrenaline induced the release of adenylyl purines from these blood vessels. The total amount of adenylyl purines released from the CA was considerably larger than that from the TA. The rank order of the amount of adenylyl purines released from the six blood vessels was $CA > SA > RA > MA > PA \geq TA$. The noradrenaline-induced release of adenylyl purines from the CA was significantly reduced by the removal of the endothelium. Noradrenaline also induced the release of adenylyl purines from cultured endothelial cells of the CA and TA. The total amount of adenylyl purines released from the former blood vessel was much larger than that from the latter. These results suggest the existence of vascular endothelial cells that are able to release ATP by an α_1 -adrenoceptor mediated mechanism, and that these cells are not homogeneously distributed in the vasculature.

Key Words: ATP-rat blood vessels-endothelial cells- α_1 -adrenoceptor-regional difference

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). There have been many reports that adenine nucleotides and nucleosides are released from a variety of sympathetically inner-

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vated tissues upon nerve stimulation. It is generally accepted that ATP was released from adrenergic nerves with noradrenaline as a co-transmitter in electrically stimulated tissues such as the vas deferens (Sneddon and Westfall, 1984) and blood vessels (Su, 1975, 1983; Sneddon and Burnstock, 1985). We have shown that the stimulation of the α_1 -adrenoceptor induced the release of ATP from the endothelium of the rat caudal artery (Westfall *et al.*, 1987; Shinozuka *et al.*, 1991, 1994). In view of the fact that ATP, ADP and adenosine have many pharmacological actions on blood vessels via purinoceptors, the endothelium-derived ATP and its metabolites may play important physiological roles in the cardiovascular system. However, there are very few reports regarding this. As an initial approach to clarify the physiological significance of ATP and its metabolites released from endothelium upon α_1 -adrenoceptor stimulation, we examined the regional differences of adenylyl purine release in several blood vessels isolated from the rat, using high-performance liquid chromatography (HPLC)-fluorescence detection.

Methods

Tissue preparations and release experiment

Male Wistar rats (SLC, Hamamatsu, Japan) ranging from 20 to 25 weeks of age were used in accordance with the Guidelines for Animal Experimentation of Shimane Medical University, which are based on the Guidelines for Animal Experimentation of the Japanese Association for Laboratory Animal Science. The caudal artery (CA), saphenous artery (SA), renal artery (RA), mesenteric artery (MA), pulmonary artery (PA) and thoracic aorta (TA) were isolated from the rats, which had been anesthetized with sodium pentobarbital (50 mg/kg intraperitoneally). These blood vessel preparations were cut open lengthwise and suspended in a water-jacketed organ chamber containing 3.0 ml of a modified Krebs solution at 37°C. The solution was continuously bubbled with 95% O₂ and 5% CO₂. The composition of the solution was as follows: (mM); NaCl 110; KCl 4.6; CaCl₂ 2.5; NaHCO₃ 24.8; KH₂PO₄ 1.2; MgSO₄ 1.2; glucose 5.6. The opened artery preparation was then allowed to equilibrate for 60 min, and the medium was replaced every 3 min during the latter half of this equilibration period. Some experiments were carried out with the opened caudal artery preparation in which the endothelial cell lining was disrupted. This was accomplished by rubbing the lumen of the arterial segment with a cotton-tipped swab.

After the 60-min equilibration period, the bathing solution was rapidly collected by draining the organ chamber every 3 min. After the first sampling (prestimulation sample), the tissues were stimulated with noradrenaline (1.0 μ M) for 3 min, and then the bathing solutions were collected (stimulation samples). The collected samples were then processed for the determination of ATP, ADP, AMP and adenosine by HPLC coupled with fluorescence detection as described by Mohri *et al.* (1993).

Cell cultures and release experiment

Endothelial cells in primary cultures from either the thoracic aorta or caudal artery of the rat were prepared by the methods previously described (Hashimoto *et al.*, 1992). The cells in

the primary cultures were incubated in 2 ml of Krebs solution for 60 min at 37°C in an atmosphere of 95% air and 5% CO₂. After 60 min of equilibration, 200 µl of bathing solution was collected (prestimulation sample) and the endothelial cells were then stimulated with noradrenaline for 3 min, followed by another 200 µl collection (stimulation sample). The collected samples were then processed for the determination of adenine nucleotides and adenosine as described previously (Mohri *et al.*, 1993).

Statistical analysis

All data are expressed as the means±S.E.M (standard error of the mean). Data were evaluated for significance by one-way analysis of variance (ANOVA) combined with Fisher's PLSD test for multiple comparison or Student's *t*-test, and the Welch test where appropriate. Values with a *P*<0.05 were considered to be significantly different. Data processing was performed with the computer software package "Stat View™ II" (Abacus Concepts, Inc., Berkeley, CA, USA).

Results

The spontaneous overflows of ATP, ADP, AMP and adenosine from the caudal artery (CA), saphenous artery (SA), renal artery (RA), mesenteric artery (MA), pulmonary artery (PA) and thoracic aorta (TA).

Fig. 1. Comparison of the spontaneous overflow of adenylyl purines from the caudal artery (CA), saphenous artery (SA), renal artery (RA), mesenteric artery (MA), pulmonary artery (PA) and thoracic aorta (TA). The vertical bars on the columns indicate the S.E.M.(n=4). Left panel (A): the spontaneous overflow amounts of each purine from the artery or aorta during a 3-min period. *t*, *d* and *m* indicate significant differences (*P*<0.05) from ATP, ADP and AMP, respectively, in each blood vessel. Right panel (B): the amounts of total purine refers to the sum total overflow of the four purines (ATP, ADP, AMP and adenosine (ADO)). *Significant difference (*P*<0.05) from CA, RA and PA. +Significant difference (*P*<0.05) from MA and TA.

and thoracic aorta (TA) are shown in Fig. 1. There were no significant differences among the four purines, such as ATP, ADP, AMP and adenosine, in CA, SA and TA (Fig. 1A). In RA and MA, the overflow amounts of ATP and ADP were significantly smaller than those of AMP and adenosine. Further, in PA, the overflow amount of AMP was largest. As shown in Fig. 1B, the total amount of adenylyl purine overflow from the SA was significantly smaller than those from the CA, RA and MA. In addition, the total amounts of purine overflow from the MA and TA were significantly smaller than that from the PA. There was no significant difference among the amounts of the overflow from the CA, RA and PA, or among those from the SA, MA and TA. In all six blood vessels, CA, SA, RA, MA, PA and TA, noradrenaline ($1\ \mu\text{M}$) significantly increased the total overflow of adenine nucleotides and nucleoside from 0.56 ± 0.15 , 0.26 ± 0.06 , 0.56 ± 0.05 , 0.37 ± 0.07 , 0.63 ± 0.08 and 0.36 ± 0.05 to 8.59 ± 0.78 , 6.25 ± 0.53 , 3.58 ± 0.40 , 1.85 ± 0.07 , 1.00 ± 0.13 and 0.46 ± 0.05 pmol/mg, respectively ($P<0.05$, paired *t*-test; $n=4$). Figure 2 shows the release of adenylyl purines from the vessels induced by noradrenaline. The release indicates the difference between the amounts of overflow of adenylyl purines in the prestimulation and stimulation samples (noradrenaline-induced overflow minus spontaneous overflow). As shown in Fig. 2A, the amounts of ATP and ADP released were significantly smaller than those of AMP and adenosine in CA, SA, RA and MA. In PA, the amount of AMP released was the largest. The total amount of purine release from the CA was significantly larger than those from the other five blood vessels (Fig. 2B). The rank order of the nora-

Fig. 2. Comparison of the noradrenaline-induced release of adenylyl purines from the caudal artery (CA), saphenous artery (SA), renal artery (RA), mesenteric artery (MA), pulmonary artery (PA) and thoracic aorta (TA). The release indicates the difference between the amount of adenylyl purines in prestimulation (in the absence of noradrenaline) and post stimulation (in the presence of noradrenaline) samples. The vertical bars on the columns indicate the S.E.M. ($n=4$). Left panel (A): the amounts of each purine released from the artery or aorta during a 3-min period. *t*, *d* and *m* indicate significant differences ($P<0.05$) from ATP, ADP and AMP, respectively, in each blood vessel. Right panel (B): the amounts of total purine which refers to the sum total of the four purines release (ATP, ADP, AMP and adenosine (ADO)). *Significant difference ($P<0.05$).

drenaline-induced amount of total adenyl purine release from the six blood vessels was $CA > SA > RA > MA > PA \geq TA$. There was no significant difference between the amounts of the release from the PA and TA. In the TA, the amount of release (pmol/mg) was only approximately 1/80 of that obtained from the CA. When the amount of release was compared on the basis of area (pmol/mm²) instead of weight of tissue (pmol/mg), the amounts of purine released by the CA, SA and RA (0.96 ± 0.08 , 0.79 ± 0.06 and 0.44 ± 0.05 pmol/mm², $n=4$, respectively) were still significantly larger than those released by the MA, PA and TA (0.17 ± 0.01 , 0.04 ± 0.01 and 0.03 ± 0.01 pmol/mm², $n=4$, respectively). In the CA, adenosine was present in the largest quantity, and was approximately equal in amount to the sum of the nucleotides ATP, ADP and AMP (Fig. 2A).

Figure 3 and 4 show the spontaneous overflow and the noradrenaline-induced release of adenine nucleotides and nucleoside from the CA in which the endothelium was left intact or was removed by rubbing the lumen, respectively. There was no significant difference between the total amounts of adenyl purine spontaneous overflow from the intact and rubbed CA (Fig. 3). However, the total amount of purine release induced by noradrenaline from the rubbed CA was significantly smaller than that from the intact CA (Fig. 4). The former was approximately 30% of the latter.

The spontaneous overflow of adenyl purines from the cultured endothelial cells which had been isolated from the CA or TA is shown in Fig. 5. There was no significant difference between the spontaneous overflow levels of adenyl purines from these cultured endothelial cells. In the cells TA and CA, noradrenaline ($10.0 \mu\text{M}$) significantly increased the overflow of total purines from 1.38 ± 0.46 and 1.46 ± 0.46 to 1.58 ± 0.46 and 4.92 ± 1.45 pmol/mg, respectively ($P < 0.05$, paired *t*-test; $n=4$). However, the noradrenaline-induced release of purine by the cultured endothelial cells from the CA was again much larger than that from the TA, with the

Fig. 3. Comparison of the spontaneous overflow of adenyl purines from intact and rubbed caudal artery of rats. The vertical bars on the columns indicate the S.E.M. ($n=4$). *Significant difference ($P < 0.05$) from each purine in intact. Left panel (A): the amounts of each purine released from the artery during a 3-min period. Right panel (B): the amounts of total purine refers to the sum total of the four purines released (ATP, ADP, AMP and adenosine (ADO)). *Significant difference ($P < 0.05$).

Fig. 4. Comparison of the noradrenaline-induced release of adenylyl purines from intact and rubbed caudal artery of rats. The vertical bars on the columns indicate the S.E.M. ($n=4$). *Significant difference ($P<0.05$) from each purine level in intact vessels. Left panel (A): the amounts of each purine released from the artery during a 3-min period. Right panel (B): the amounts of total purine refers to the sum total of the four purines released (ATP (B), ADP, AMP and adenosine (ADO)). *Significant difference ($P<0.05$).

Fig. 5. Comparison of the spontaneous overflow of adenylyl purines from the endothelial cells in primary culture. Cells were prepared from the caudal artery (CA) and thoracic aorta (TA) of rats. The vertical bars on the columns indicate the S.E.M. ($n=4$). Left panel (A): the overflow amounts of each purine from the artery or aorta during a 3-min period. Right panel (B): the amounts of total purine refers to the sum total overflow of the four purines (ATP, ADP, AMP and adenosine (ADO)).

former approximately 10 times the latter (Fig. 6). This release of purines from specific endothelial cell populations is therefore in agreement with the results obtained with intact CA and TA. As shown in Fig. 6A, the amount of ATP (1.95 ± 0.68 pmol/10⁴ cells) released from cultured endothelial cells from the CA by noradrenaline was significantly ($P<0.05$) larger than that of adenosine (0.03 ± 0.02 pmol/10⁴ cells).

Fig. 6. Comparison of spontaneous overflow and noradrenaline-induced release of adenylyl purines from endothelial cells in primary culture. Cells were prepared from the caudal artery (CA) and thoracic aorta (TA) of rats. The vertical bars on the columns indicate the S.E. M. (n=4). *Significant difference ($P < 0.05$) from each purine level in CA. Left panel (A): the amounts of each purine released from the artery or aorta during a 3-min period. Right panel (B): the amounts of total purine refers to the sum total of the four purines released (ATP, ADP, AMP and adenosine (ADO)).

Discussion

Previous study at our laboratories (Shinozuka *et al.*, 1994) has indicated that noradrenaline released ATP and its metabolites via the stimulation of α_1 -adrenoceptors in the rat caudal artery. The present experimental results also suggest that a functional role exists for an α_1 -adrenoceptor-mediated release of adenylyl purines that is regionally differentiated in the vasculature. The rank order of the amounts of total adenylyl purines released by noradrenaline from the six blood vessels was caudal artery > saphenous artery > renal artery > mesenteric artery > renal artery $\geq$ thoracic aorta. These release values were compared on the bases of both pmol/mg wet weight and pmol/mm² area of tissue analyzed. The differences among the amount of purines released from the six blood vessels thus do not appear to be due to differences in the area of the intima layer (i.e., the number of endothelial cells).

The present results demonstrated that 1) the release of purines from the caudal artery was reduced by rubbing the lumen of the artery, and that 2) noradrenaline induced the release of purines in cultured endothelial cells from the caudal artery and thoracic aorta, and the amount of release from the former was larger than that from the latter. Taken together, these findings suggest that the vascular endothelial cells are able to release adenylyl purines by an α_1 -adrenoceptor-mediated mechanism and seem not to be homogeneously distributed in the vasculature. As one possibility, the density and the mode of distribution of the α_1 -adrenoceptor on the endothelial cells mediating adenylyl purine release may be different in the vasculature.

The adenylyl purines released from the primary cultures of endothelial cells from the caudal artery of rats by noradrenaline were mostly nucleotides, mainly ATP. However, in intact caudal artery preparations, the amount of adenosine released was 46% of the total amount of adenylyl purines released. It has been reported that the quantity of adenine nucleotides released

from cultured endothelial cells was larger than the amount of adenosine released, and it was suggested that the degradation of ATP to adenosine by endothelial cell ectonucleotidase is not very rapid (Pearson and Gordon, 1979; Gordon *et al.*, 1986). In contrast, those investigators found that ATP is rapidly degraded to adenosine by cultured smooth muscle cells from the pig aorta (Gordon *et al.*, 1989). We showed that the amount of ATP released by noradrenaline from the intact caudal artery of rats was increased approximately 2-fold by the 5'-nucleotidase inhibitor, α , β -methylene ADP (Shinozuka *et al.*, 1994). Thus, most of the ATP released from endothelial cells by noradrenaline may be rapidly hydrolyzed to adenosine by ectonucleotidase on the surface of smooth muscle cells. In primary cultures of rat caudal artery smooth muscle cells, we found no significant release of purines induced by noradrenaline (data not shown). Bodin *et al.* (1991) have also reported that shear stress did not evoke ATP release in cultured vascular smooth muscle cells. These findings suggest that the vascular smooth muscle cells may not possess a significant ATP releasing function, but may be important in the metabolic conversion of ATP to adenosine. In the present study, we observed that the composition of purines released differed somewhat depending on the region of blood vessel. As one possible explanation for this difference, it is considered that the distribution and/or activity of metabolic converting enzyme, for example ectonucleotidase, is not homogenous in the vasculature.

Taken together, our findings confirm that an α_1 -adrenoceptor-mediated mechanism for ATP release exists on the vascular endothelial cells, and the data strongly suggest that 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 six rat blood vessels. ATP and ADP can produce endothelium-dependent vasodilatation via P_{2Y} -purinoceptors in the precontracted arteries, and the metabolite of ATP, adenosine, can also produce direct vasodilatation by cAMP accumulation in the arterial smooth muscle cells via $P_1(A_2)$ -purinoceptor (Dalziel and Westfall, 1994). Both ATP and adenosine reduced noradrenaline release from vascular sympathetic nerves via P_3 -purinoceptors (Shinozuka *et al.*, 1991). Endogenous adenylyl purines released from the arteries may thus produce vasodilatation via P_{2Y} -, $P_1(A_2)$ -, and P_3 -purinoceptor stimulation, and might control the blood circulation and blood pressure. Indeed, we have shown that ATP and its metabolites released from vascular endothelium are related to the control of blood pressure (Hashimoto *et al.*, 1995). Based on these observations, it is suggested that the regional difference of endothelium-derived ATP release may be associated with the control of the local blood circulation and blood pressure.

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