The effects of age on the release of adenine nucleosides and nucleotides from rat caudal artery

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1. The spontaneous and α-adrenoceptor-induced release of ATP, ADP, AMP and adenosine were determined from arterial segments and from isolated endothelial cells from caudal arteries of young (5-week-old), adult (30-week-old) and old (100- to 110-week-old) Wistar rats.

2. The spontaneous (non-evoked) release of the sum total of the four purines was significantly greater from artery segments of young rats than from adult and old rats.

3. The release of the adenine nucleotides and adenosine induced by methoxamine (10 μM), an α_1-adrenoceptor agonist, was greater from artery segments from young rats than from old rats.

4. The spontaneous release of the sum total of the four purines was significantly greater from endothelial cells prepared from caudal arteries of young rats than of old rats.

5. The noradrenaline (10 μM)-induced release of the sum total of the four purines was significantly greater from endothelial cells prepared from caudal arteries of young rats than of old rats.

6. The levels of adenine nucleotides and adenosine, determined in plasma from anaesthetized rats, were significantly higher in young rats compared with adult and old rats.

7. These findings suggest that the release of ATP from the vascular endothelial cells is reduced with advancing age.

Adenosine triphosphate (ATP), in addition to its well-known role in intracellular metabolism, is increasingly being recognized for its extracellular actions in various tissues including blood vessels (Gordon, 1986; Burnstock, 1991; Dalziel & Westfall, 1994). Perhaps the best recognized function of extracellular ATP is as a cotransmitter with noradrenaline (NA) when released from postganglionic sympathetic nerves (Westfall, Sedaa, Shinozuka, Bjur & Buxton, 1990). However, nerves are not the only cell type that can release ATP and its congeners. For example, adenine nucleosides and nucleotides are released from extraneuronal sites of *in vitro* blood vessel preparations as a consequence of α_1-adrenoceptor stimulation by methoxamine (Westfall, Sedaa & Bjur, 1987; Sedaa, Bjur, Shinozuka & Westfall, 1990; Shinozuka, Sedaa, Bjur & Westfall, 1991). The major source of the purines released by α-adrenoceptor stimulation of blood vessels appears to be the endothelium (Sedaa *et al.* 1990). Indeed subsequent work has shown ATP to be released from cultured cardiac endothelial cells by NA (Westfall *et al.* 1990) as well as by a number of other vasoactive substances such as bradykinin, acetylcholine and serotonin (Yang, Cheek, Westfall & Buxton, 1994). Physical stimuli, such as shear stress, are also known to release ATP from cultured endothelial cells (Bodin, Bailey & Burnstock, 1991).

There appear to be a number of ways in which extracellular adenine nucleotides and adenosine can influence vascular function. Obviously one important way is when ATP is released as a transmitter from sympathetic nerves and acts on P_{2X}-purinoceptors on vascular smooth muscle cells to cause muscle contraction and vasoconstriction. There are other vascular actions as well. Shinozuka *et al.* (1991) have shown that adenine nucleosides and nucleotides released from extraneuronal sites in blood vessels can act on prejunctional purinoceptors on sympathetic nerves to

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modulate the release of transmitters. ATP derived from endothelial cells or other sources can act on P_{2Y}-purinoceptors on endothelial cells to evoke the release of endothelium-derived relaxing factor (EDRF), which causes relaxation of vascular smooth muscle (De May & Vanhoutte, 1981; De May, Claeys & Vanhoutte, 1982; Burnstock & Kennedy, 1985). The adenosine that is formed from the metabolism of adenine nucleotides extracellularly can produce vasodilatation by acting on P_1-purinoceptors on vascular smooth muscle (Burnstock, 1987). Burnstock proposed that during ischaemia, ATP is released from the endothelium, which then produces vasodilatation through P_{2Y}- and P_1-purinoceptors. In contrast to such a pathophysiological role, ATP released from endothelium via α_1-adrenoceptor stimulation might physiologically control local blood flow in response to sympathetic nervous system activity.

These effects of ATP and adenosine have been shown to change with advancing age. Some investigators have shown that endothelium-dependent relaxation induced by ATP (Chinellato *et al.* 1991) and adenosine (Moritoki, Matsugi, Takase, Ueda & Tanioka, 1990) were reduced with advancing age. On the other hand, Koga, Takata, Kobayashi, Fujii, Nagao & Fujishima (1992) suggested that ageing may activate a P_2-purinoceptor leading to the generation of endothelium-derived relaxing factor. Lüscher & Noll (1993) reviewed the evidence that the release of nitric oxide decreases with age but the production of endothelin-1 remains stable or increases. These findings led us to the hypothesis that α-adrenoceptor-induced release of ATP from the vascular endothelium may be reduced with an increase in age. Recently, substances derived from endothelial cells have been recognized as taking part in the regulation of vascular tone (Furchgott & Vanhoutte, 1989; Moncada, Palmer & Higgs, 1991; Rubanyi, 1993). An alteration of ATP release could participate in an elevation in the blood pressure associated with ageing. To test this notion, we examined the effect of rat ageing on α_1-adrenoceptor-induced release of ATP from the caudal artery and the cultured endothelial cells, and also compared the plasma ATP levels of rats of different ages.

METHODS

Systolic blood pressure and plasma adenine nucleotides and adenosine

Rats used in this study were provided and killed in accordance with the procedures outlined in the Guidelines for Animal Experimentation of Shimane Medical University (see below), which was compiled from the Guidelines for Animal Experimentation of the Japanese Association for Laboratory Animal Science. Rats were kept at an environmentally controlled room temperature of 23 ± 2 °C and relative humidity of 50 ± 10%, with automatic lighting from 08.00 to 20.00 h.

The blood pressure and plasma adenine nucleotides and adenosine of young (5-week-old), adult (30-week-old) and old (100- to 110-week-old) female Wistar rats were measured. After measuring systolic and mean blood pressure (P_S and P_M) by the tail-cuff plethysmographic method (Ueda, UR-1000, Tokyo, Japan), the diastolic blood pressure (P_D) was calculated from the following equation:

$$P_D = (3P_M - P_S)/2,$$

where P_S and P_M were measured by plethysmography.

Following the measurement of blood pressure, rats were anaesthetized with sodium pentobarbitone (50 mg ml^{-1}) and the blood was collected into heparinized syringes. The blood was collected into a polyethylene tube containing EDTA (1 mM) to prevent clotting and was centrifuged for 20 min at 3000 r.p.m. at 4 °C. After being checked for platelet contamination with an automated haematology analyser (K-2000, Toa Medical Electronics Co. Ltd, Kobe, Japan) ($< 10^3$ μl^{-1}), the resulting plasma (20 μl) was assayed (by HPLC fluorescence detection) for its content of ATP, ADP, AMP and adenosine.

Tissue preparation and release experiments

Young, adult and old female Wistar rats were anaesthetized with sodium pentobarbitone (50 mg kg^{-1} I.V.) and killed by exsanguination. As long a segment as possible (approximately 8–13 cm, 20–30 mg wet weight) of the caudal artery was removed, cleaned of connective tissue and suspended in a water-jacketed organ chamber containing 2·0 ml of a modified Krebs solution at 37 °C. The solution was continuously bubbled with 95% O_2–5% CO_2. The composition of the solution was as follows (mM): NaCl, 110; KCl, 4·6; $CaCl_2$, 2·5; $NaHCO_3$, 24·8; KH_2PO_4, 1·2; $MgSO_4$, 1·2; glucose, 5·6. The artery was allowed to equilibrate for 60 min, and the medium was replaced every 3 min during the latter half of the equilibration period.

After the 60 min equilibration period, the bathing solution was collected by draining the organ chamber every 3 min. After the first sampling (which was used to determine spontaneous release) the tissue was stimulated with methoxamine (10 μM) for 3 min and the bathing solution (stimulation sample) was collected. The samples were processed for the determination of ATP, ADP, AMP and adenosine by high-performance liquid chromatography (HPLC) fluorescence detection as described in detail previously (Mohri, Takeuchi, Shinozuka, Bjur & Westfall, 1993).

Cell culture of endothelial cells from rat caudal artery and release experiments

Caudal arteries of young (5-week-old) and old (100- to 110-week-old) female Wistar rats were prepared and a primary culture of endothelial cells was established according to the method previously described (Hashimoto, Hara, Honda, Ishinaga, Moriyama & Masumura, 1992). Briefly the rats were anaesthetized with diethyl ether and immediately killed by exsanguination. The caudal arteries were excised from the rats, and were rinsed five times in Dulbecco's modified Eagle's medium (DMEM) which contained antibiotics (100 u ml^{-1} penicillin, 100 μg ml^{-1} streptomycin) and an antifungal agent (0·25 μg ml^{-1} fungizone). After removal of the adipose and connective tissue from the adventitial surface of the vessels and with care taken not to damage the endothelium, the arteries were cut into flat segments of approximately 2 mm^2. Each segment was placed endothelial side down into wells of collagen plates (Celltight C-1, Sumitomo Bakelite Co. Ltd, Osaka, Japan), and just enough growth medium containing 50 μg ml^{-1} endothelial cell growth factor (ECGF; Boehringer-Mannheim, Mannheim, Germany) and 3% fetal bovine serum (FBS; Gibco BRL, Grand Island, NY, USA) in Opti-MEM® 1 (Gibco)) was added to keep the tissue piece moist. The

Table 1. Body weight and systolic blood pressure of young, adult and old rats

	n	Body weight (g)	P_S (mmHg)	P_D (mmHg)
Young rat	11	79·1 ± 1·28*	104 ± 2·13*	46·9 ± 4·02*
Adult rat	9	285 ± 9·21†	149 ± 4·08†	102 ± 4·27†
Old rat	9	407 ± 18·9‡	167 ± 5·43‡	125 ± 7·09‡

Young rats, 5 weeks old; adult rats, 30 weeks old; old rats, 100- to 110 weeks old. P_S, systolic blood pressure; P_D, diastolic blood pressure. Values are expressed as means ± S.E.M.; n, number of rats tested. *, †, ‡, means in the same column not sharing a common identification mark are significantly different ($P < 0·05$).

arterial segments were incubated in a humidified incubator at 37 °C in a 95% air–5% CO_2 atmosphere.

Once the endothelial cells proliferated, usually by about 48 h, the vascular muscle segments were removed from the culture plates leaving the endothelial cells behind and the growth medium was exchanged with renewal medium (25 μg ml^{-1} ECGF, 3% FBS and 5 u ml^{-1} of heparin in DMEM). The remaining cells were cultivated for a further 5 days with the renewal medium being exchanged every 2 days, and after having grown to confluent monolayers the cells were used for release experiments of adenine nucleotides and adenosine.

To identify endothelial cells in primary culture, immunocytochemical characterization was performed using a monoclonal antibody against Factor VIII-related antigen (a Biomeda Histo Scan Kit, Cosmo Bio Co. Ltd, Tokyo, Japan) and using acetylated low density lipoprotein labelled with the fluorescent probe 1,1′-dioctadecyl-1-3,3,3′,3′-tetramethyl-indocarbocyanine perchlorate (DiI-Ac-LDL; Biomedical Technologies, Inc., Stoughton, MA, USA), as previously described (Hashimoto *et al.* 1992).

To evaluate the release of purines, the endothelial cells in primary culture (30×10^4 to 35×10^4 cells, 35 mm dish for young rats, 20×10^4 to 25×10^4 cells, 35 mm dish for old rats) in collagen dishes were washed in the modified Krebs solution and then incubated in 2 ml of the solution for 60 min at 37 °C in a 95% air–5% CO_2 atmosphere. After this equilibration period, 200 μl of the bathing solution was collected (prestimulation sample) and the endothelial cells were stimulated with noradrenaline (10 μM) for 3 min, and the solution (stimulation sample) collected. The collected solution was then processed for the determination of ATP, ADP, AMP and adenosine by HPLC fluorescence detection as described for the experiments with caudal arteries.

Statistical significance

Data were expressed as means and standard errors of the mean (S.E.M.). A statistical analysis was performed by one-way analysis of variance (ANOVA) and the Student–Newman–Keuls test for multiple comparison (Table 1, Figs 1, 2 and 5) and by Student's t test for simple comparison (Figs 3 and 4). Values with a $P < 0·05$ were considered to be significantly different.

RESULTS

Body weight and systolic and diastolic blood pressure

Table 1 shows the mean body weight and mean systolic and diastolic blood pressure of the three groups of rats used. As expected, the body weights of the rats increased with age. Systolic and diastolic blood pressure also increased with age. Adult rats had significantly higher blood pressures than young rats. Old rats, in turn, had significantly higher blood pressures than adult rats.

Spontaneous release of adenine nucleotides and adenosine from caudal arteries

Figure 1 illustrates the spontaneous release of the adenine nucleotides and nucleosides from caudal arteries of the three age groups of Wistar rats. The spontaneous release of the sum total of the purines from caudal arteries of young rats was significantly higher than that from arteries of adult or old rats. The release of AMP was significantly higher in arteries from young rats and the release of ATP and ADP from arteries of young rats, while exhibiting a higher mean value, was not significantly different from the

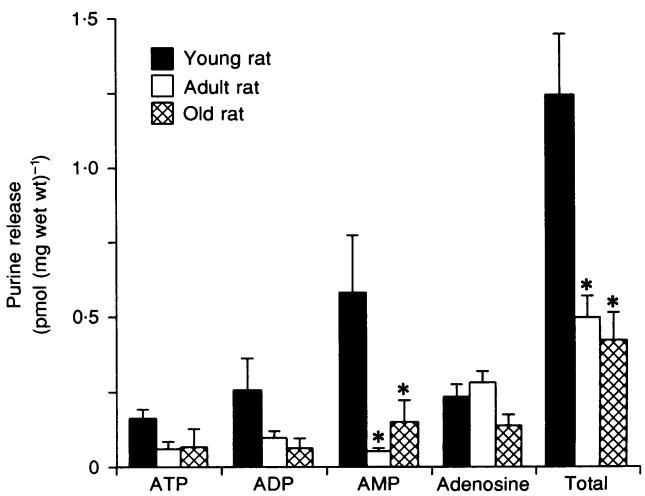

Figure 1. Comparison of the spontaneous release of adenine nucleotides and adenosine from the caudal arteries of young, adult and old rats

Shown are the amounts of each purine released from the caudal artery during 3 min. In this and subsequent figures 'total' refers to the sum total of the four purines, ATP, ADP, AMP and adenosine, and vertical bars on the columns indicate S.E.M. * Statistically significant difference from young rats ($P < 0·05$); $n = 8$.

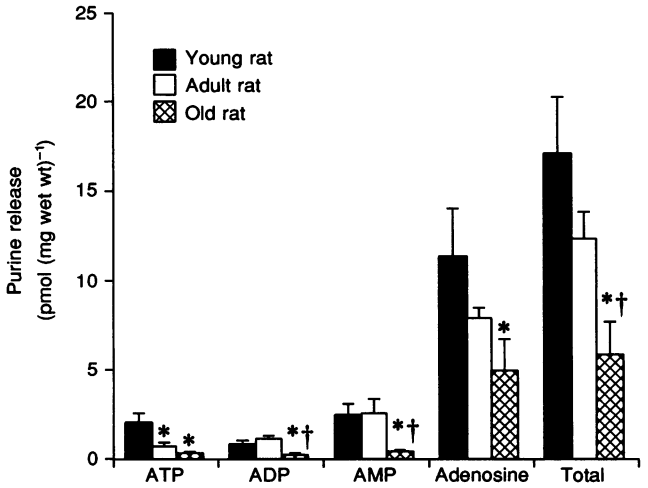

Figure 2. Comparison of the methoxamine (10 μM)-induced release of adenine nucleotides and adenosine from the caudal arteries of young, adult and old rats

* Statistically significant difference from young rats ($P < 0{\cdot}05$). † Statistical significance between adult and old rats ($P < 0{\cdot}05$); $n = 8$.

other age groups. The spontaneous release of adenosine was not different among the three groups.

Methoxamine-induced release of adenine nucleotides and adenosine from caudal arteries

As previously reported (Shinozuka *et al.* 1991) the effect of the α-adrenoceptor agonist methoxamine on the release of adenyl purines from the caudal artery was examined, and a large amount of purine release was observed (Fig. 2). The release of purines induced by methoxamine was abolished in the presence of 0·03 μM bunazosin, an α_1-receptor antagonist (data not shown).

The methoxamine-induced release of the sum total of the purines was highest in arteries from young rats and lowest in arteries from old rats, with release from adult animals being at an intermediate level. Although there are some individual differences, the pattern of a greater methoxamine-induced release from arteries of young rats was generally seen with the individual nucleotide and nucleoside compounds.

Spontaneous and α-adrenoceptor-induced release of adenine nucleotides and adenosine from cultured caudal artery endothelial cells

In addition to methoxamine, noradrenaline (NA) at 1·0 and 10·0 μM also elicited the release of adenine nucleotides and adenosine from the caudal artery of adult rats, which was abolished in the presence of bunazosin at 30 nM. The amount of purine release induced by NA at 10 μM was significantly larger (20·1 $\pm$ 3·4 pmol mg^{-1}, $n = 4$) than that of methoxamine at the same concentration (10·4 $\pm$ 1·4 pmol mg^{-1}, $n = 5$). Therefore, NA was used as an α_1-adrenoceptor stimulant in the release experiment of cultured endothelial cells.

Endothelial cells from the endothelial surface of caudal arteries of young and old rats were cultured according to the culture method previously used in rat thoracic aortae (Hashimoto *et al.* 1992). These cells, which had achieved confluency in the culture well, exhibited a cobblestone morphology typical of cultured endothelial cells and had the ability to incorporate fluorescent DiI-Ac-LDL and also

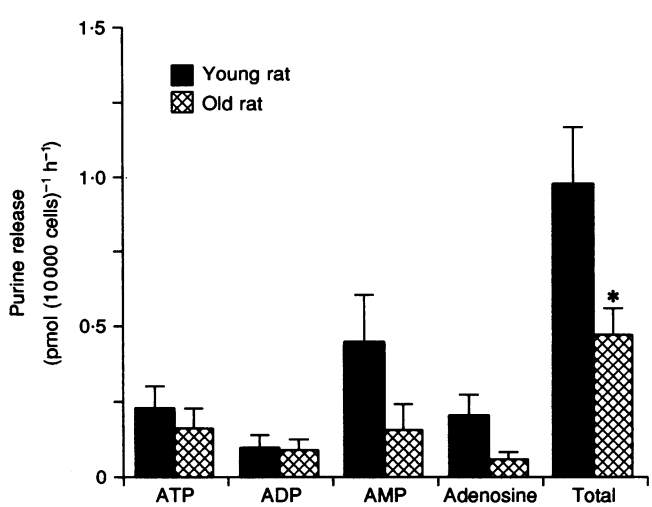

Figure 3. Spontaneous release of adenine nucleotides and adenosine from endothelial cells in primary culture

Cells were prepared from caudal arteries of young (5-week-old; $n = 8$) and old (100- to 110-week-old; $n = 7$) rats. Shown are mean values of the purines that are released from the endothelial cells over a period of 60 min. * Statistically significant difference between cells of young and old rats.

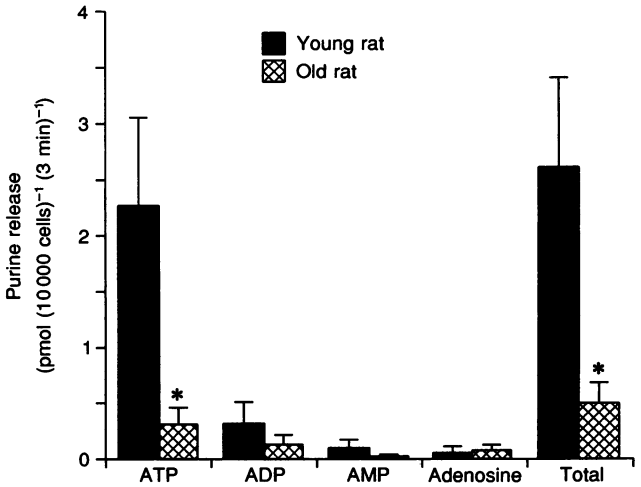

Figure 4. The effect of noradrenaline (10 μM) on the release of adenine nucleotides and adenosine from endothelial cells in primary culture prepared from arteries of young and old rats

* Statistically significant difference between cells of young ($n = 8$) and old ($n = 7$) rats.

to stain positive for the Factor VIII-related antigen (Hashimoto *et al.* 1992). Because of these characteristics, we believe our technique is suitable for generating authentic endothelial cells.

Figure 3 shows the spontaneous release of the adenine nucleotides and nucleosides from endothelial cells cultured from caudal arteries of rats of various ages. The spontaneous release of the sum total of the purines was twice as great from cells prepared from young rats as that from old rats.

Figure 4 shows the effect of stimulation of endothelial cells with NA on the release of purines. The NA-evoked release of the sum total of the purines was significantly greater in cells from arteries of young animals than from old animals. This effect of NA on total purine release is apparently due primarily to an effect on the release of ATP.

Plasma levels of adenine nucleotides and adenosine

Figure 5 shows the plasma levels of individual adenine nucleotides and nucleosides and the sum total of these purines. Young rats exhibited a higher concentration of purines in their plasma than either adult or old rats. These differences in total purines are related primarily to higher plasma levels of ATP and ADP.

The relation between blood pressure and adenyl purines

The relationship between blood pressure and the amounts of adenyl purines released *in vitro* was also examined. A regression analysis of ATP and total purines (TP) released from the caudal artery, both spontaneous and methoxamine induced, and systolic and diastolic blood pressure (P_S and P_D) of rats in the three age groups revealed a significant inverse relationship. That is, the blood pressure was highest in animals in which the tissues released the lowest amount of purines (spontaneous ATP and TP *vs.* P_S: $r = -0{\cdot}948$ and $-0{\cdot}979$, respectively; methoxamine-induced ATP and TP *vs.* P_S: $r = -1{\cdot}000$ and $-0{\cdot}948$, respectively; spontaneous ATP and TP *vs.* P_D: $r = -0{\cdot}929$ and $-0{\cdot}958$, respectively; methoxamine-induced ATP and TP *vs.* P_D: $r = -1{\cdot}000$ and $-0{\cdot}920$, respectively).

A regression analysis of plasma ATP and total plasma purine content *versus* the P_S and P_D also revealed a

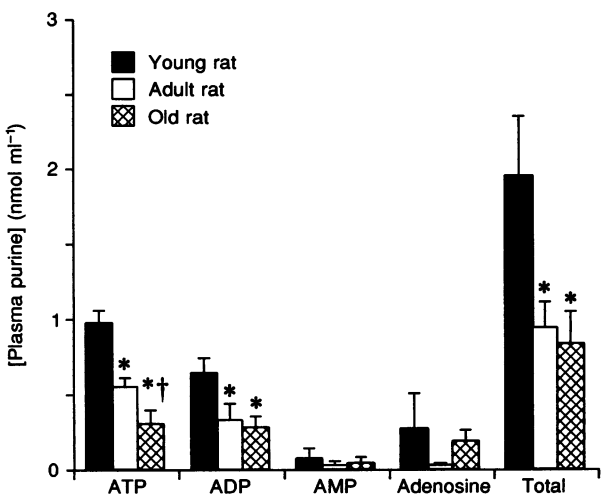

Figure 5. Comparison of plasma concentrations of adenine nucleotides and adenosine in young, adult and old rats

Each bar represents the mean value from 6 rats.
* Statistically significant difference from young rats ($P < 0{\cdot}05$). † Statistically significant difference between adult and old rats ($P < 0{\cdot}05$).

significant inverse relationship. That is, blood pressure was highest when plasma purines were lowest (ATP and TP *vs.* P_S: $r = -0{\cdot}993$ and $-0{\cdot}989$, respectively; ATP and TP *vs.* P_D: $r = -0{\cdot}997$ and $-0{\cdot}980$, respectively).

DISCUSSION

Our previous studies with rat caudal artery demonstrated that both electrical nerve stimulation and methoxamine, an α_1-adrenoceptor agonist, produced a large release of adenine nucleotides and adenosine (Westfall *et al.* 1987; Shinozuka *et al.* 1991). In the present study, we confirmed the considerable release of adenyl purines induced by methoxamine. Also, we observed that NA produced a larger amount of purine release than methoxamine. As one explanation, both α_1- and α_2-adrenoceptors might participate in the release of purines from the caudal artery of rats in a synergystic manner. However, this is unlikely since the NA-induced release of purines was blocked by bunazosin, an α_1-adrenoceptor antagonist, but not by idazoxan, an α_2-adrenoceptor antagonist (Shinozuka, Hashimoto, Masumura, Bjur, Westfall & Hattori, 1994*a*). Oshita, Kigoshi & Muramatsu (1993) suggested that, in rabbit thoracic aorta, NA produced contraction via two distinct α_1-adrenoceptors, α_{1H} and α_{1L}. They also showed that methoxamine produced the contraction via α_{1L}, and showed that the EC_{50} of the contractile response for NA was smaller than that for methoxamine. This may indicate that the NA-induced purine release is produced through two distinct α_1-adrenoceptors.

The amounts of nucleotides released by α_1-adrenoceptor stimulation of intact caudal artery segments was smaller than that of adenosine. However, in primary cultures of endothelial cells from the caudal artery, the amount of ATP released by NA exceeded that of its metabolites, ADP and AMP, and adenosine. We have not yet collected sufficient data to explain this difference. Pearson & Gordon (1979) also showed that the quantity of adenine nucleotides released from cultured endothelial cells was greater than that of adenosine. Slakey & Gordon (1990) suggested that ATP is rapidly degraded to adenosine by cultured smooth muscle cells, but slowly degraded by endothelial cells from pig aorta. As one possible explanation, 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 the case of rubbed caudal artery, NA-induced release of purines was significantly decreased by rubbing the lumen, and this indicates that the source of the purines may be the endothelium. More recently, we have observed that NA did not produce a release of purines from cultured smooth muscle cells from the caudal artery of rats (Shinozuka *et al.* 1994*a*). Bodin *et al.* (1991) also reported that shear force did not evoke ATP release in cultured smooth muscle cells, although it did cause the release in cultured endothelial cells. Probably the main source of ATP release is via α_1-adrenoceptor stimulation of the endothelium. The exact reason why the release of purines was not abolished in the denuded caudal artery is unknown. As one possibility, we can point out that some endothelial cells may remain after rubbing, because it is very difficult to remove entirely all endothelial cells among a very thin caudal artery of 12 cm in length.

An important new observation of the present study is that the α_1-adrenoceptor-induced release of the adenyl purines from the caudal artery decreases as the rats age. This inverse relationship between purine release and age extended to cultured endothelial cells prepared from caudal arteries of rats of various ages. Thus, endothelial cells prepared from arteries of young rats released significantly more purines both spontaneously and upon α_1-adrenoceptor stimulation than endothelial cells from old rats. Recently, we have shown that there was no significant difference between the amount of total purines released by the α_1-adrenoceptor agonist methoxamine from the caudal artery of spontaneously hypertensive rats (20 to 25 weeks old) and age-matched normotensive Wistar Kyoto rats (Shinozuka, Kunitomo, Hattori, Bjur & Westfall, 1995). Therefore, this age-related decrease in the release of adenyl purines from rat caudal arteries may not be caused by high blood pressure. Generally endothelial cell function is reduced on ageing (Lüscher & Noll, 1993). It is not clear whether this age-related decrease in the release of purines from endothelial cells is a generalized feature of ageing *per se*. There is at least one known situation in which ATP release from endothelial cells is enhanced with age. Milner, Bodin, Loesch & Burnstock (1992) have shown that the shear stress-induced release of ATP from isolated endothelial cells of rabbit thoracic aorta is increased in cells from older rats.

The reason why α_1-adrenoceptor-stimulated release of adenine nucleotides and nucleosides decreases with age is not known at this time. However, this phenomenon may be related to an alteration in density and/or affinity of adrenoceptors. There are many reports on the age-related impairments in α-adrenergic responsiveness (Docherty, 1990). Although this issue remains poorly described, there are reports that the contractile response of the caudal artery to α-adrenoceptor stimulants decreases with age, an effect that may be due to a decrease in the density and/or affinity of α_1-adrenoceptors on the vascular smooth muscle (Fouda & Atkinson, 1986). One possibility is that the loss of α_1-adrenoceptor responsiveness of the smooth muscle of the caudal artery extends to the endothelial cells of the blood vessel as well.

On the other hand, there are some reports that the ATP content in the heart (Finelli *et al.* 1993) and liver (de la Cruz, Buron & Roncero, 1990) of rats was lower in rats aged 22–24 months. Also, Yamaguchi, Ozaki & Suketa (1990) reported that the ATP content in cultured bone

tissue from 30-week-old rats fell in comparison with that from 3-week-old rats. Tummino & Gafni (1991) proposed a possible age-related decrease in the maximal rate of ATP synthesis in rat liver. These observations indicate a second possibility that the supply of ATP for α_1-adrenoceptor-coupled release may be decreased with advancing age.

There are other age-related changes in blood vessels that could contribute to a decreased release of purines. The accumulation of calcium salts and lipids are the most striking and consistent age-related changes in arteries (Kohn, 1977). As rats age, plasma cholesterol rises, as does the cholesterol and fatty acid content of arteries (Uchida, Nomura, Kadowaki, Takase, Takano & Takeuchi, 1978), and therefore one might expect membrane fluidity to be altered. Changes in the membrane fluidity of endothelial cells has been reported to influence the release of EDRF (Shimokawa & Vanhoutte, 1986). Furthermore, in a recent study (Shinozuka *et al.* 1994*b*), we reported that ATP release from the endothelium mediated via α_1-adrenoceptors is impaired in the caudal artery of rats with arteriosclerosis and that this is correlated with increased calcification and an increase in cholesterol content. As a third possibility, perhaps changes in membrane fluidity and/or the calcification that is associated with ageing may lead to alterations in α_1-adrenoceptor-induced release of ATP and related adenine compounds from endothelial cells.

While the ATP that is released from sympathetic nerves at the neuroeffector junction in blood vessels appears to play a role in mediating vasoconstriction, the large amounts of ATP and related adenine compounds released from endothelial cells would seem to be more involved in producing vasodilatation. ATP, for example can cause vasodilatation by releasing EDRF from endothelial cells (DeMey & Vanhoutte, 1981). The present findings suggest that most of the ATP released from the endothelium is metabolized to adenosine in the smooth muscle of rat caudal artery. Adenosine, released as such and that which is formed by the extracellular metabolism of adenine nucleotides, can cause vasodilatation via a direct action on vascular smooth muscle (Burnstock, 1987). Also both adenosine and adenine nucleotides can reduce the action potential-induced release of neurotransmitters from sympathetic nerves (Shinozuka, Bjur & Westfall, 1988). Furthermore, we have demonstrated that adenyl purines released by α_1-adrenoceptor stimulation inhibited the release of NA induced by electrical field stimulation in the caudal artery of rats (Shinozuka *et al.* 1991, 1995). The concentration of endogenous adenyl purines may be in the range to effectively interact with purinoceptors of vascular tissues (Shinozuka *et al.* 1994*a*). In these manners, endothelial-derived purines may act as autocrine and paracrine substances that influence blood vessel tone as well as other aspects of blood vessel function (Yang *et al.* 1994).

In this context, it is of interest that both the spontaneous release and the α_1-adrenoceptor-stimulated release of ATP and related purines is less from blood vessels and cultured endothelial cells from older animals. Furthermore, the plasma levels of purines are correlated inversely with the rise in systolic and diastolic blood pressures that accompanies ageing in the rats. ATP and its metabolite adenosine may play a role in the regulation of blood pressure by their various actions on the cardiovascular system (Burnstock, 1988). ATP release induced by α_1-adrenoceptor stimulation from the vascular endothelium may participate in changes of the blood pressure with ageing.

Bodin, P., Bailey, D. & Burnstock, G. (1991). Increased flow-induced ATP release from isolated vascular endothelial cells but not smooth muscle cells. *British Journal of Pharmacology* **103**, 1203–1205.

Burnstock, G. (1987). Local control of blood pressure by purines. *Blood Vessels* **24**, 156–160.

Burnstock, G. (1988). Local purinergic regulation of blood pressure. In *Vasodilatation: Vascular Smooth Muscle, Peptides, Autonomic Nerves, and Endothelium*, ed. Vanhoutte, P. M., pp. 1–14. Raven Press, New York.

Burnstock, G. (1991). Distribution and roles of purinoceptor subtypes. *Nucleosides and Nucleotides* **10**, 917–930.

Burnstock, G. & Kennedy, C. (1985). Is there a basis for distinguishing two types of P_2-purinergic receptor? *General Pharmacology* **16**, 433–440.

Chinellato, A., Pandolfo, L., Ragazzi, E., Zambonin, M. R., Froldi, G., De Biasi, M., Caparrotta, L. & Fassina, G. (1991). Effect of age on rabbit aortic responses to relaxant endothelium-dependent and endothelium-independent agents. *Blood Vessels* **28**, 358–365.

Dalziel, H. H. & Westfall, D. P. (1994). Receptors for adenine nucleotides and nucleosides: Subclassification, distribution, and molecular characterization. *Pharmacological Reviews* **46**, 449–466.

De La Cruz, J., Buron, I. & Roncero, I. (1990). Morphological and functional studies during aging at mitochondrial level. Action of drugs. *International Journal of Biochemistry* **22**, 729–735.

De Mey, J. G., Claeys, M. & Vanhoutte, P. M. (1982). Endothelium-dependent inhibitory effects of acetylcholine, adenosine triphosphate, thrombin and arachidonic acid in the canine femoral artery. *Journal of Pharmacology and Experimental Therapeutics* **222**, 166–173.

De Mey, J. G. & Vanhoutte, P. M. (1981). Role of the intima in cholinergic and purinergic relaxation of isolated canine femoral arteries. *Journal of Physiology* **316**, 347–355.

Docherty, J. R. (1990). Cardiovascular responses in ageing: a review. *Pharmacological Reviews* **42**, 103–125.

Finelli, C., Aussedat, J., Ray, A., Lortet, S., Lavanchy, N., Guarnieri, C., Caldarera, C. M. & Rossi, A. (1993). Effect of age on phosphorylated compounds and mechanical activity of isolated rat heart: a ^{31}P-NMR study. *Cardiovascular Research* **27**, 1978–1982.

Fouda, A.-K. & Atkinson, J. (1986). Sensitivity to noradrenaline and electrical stimulation decreases with age in the rat tail artery. *Naunyn–Schmiedeberg's Archives of Pharmacology* **334**, 37–39.

Furchgott, R. F. & Vanhoutte, P. M. (1989). Endothelium-derived relaxing and contracting factors. *FASEB Journal* **3**, 2007–2018.

Gordon, J. L. (1986). Extracellular ATP: effects, sources and fate. *Biochemical Journal* **23**, 303–319.

Hashimoto, M., Hara, T., Honda, M., Ishinaga, Y., Moriyama, K. & Masumura, S. (1992). Metabolic aspects of endothelial cells cultured from rat aortae. *Artery* **19**, 284–296.

Koga, T., Takata, Y., Kobayashi, K., Fujii, K., Nagao, T. & Fujishima, M. (1992). Age-related changes in P_2-purinergic receptors on vascular smooth muscle and endothelium. *Hypertension* **19**, 286–289.

Kohn, R. R. (1977). Heart and cardiovascular system. In *Handbook of the Biology of Aging*, ed. Finch C. E. & Hayflick L., pp. 281–317. Van Nostrand Reinhold Company, New York.

Lüscher, F. T. & Noll, G. (1993). Endothelium-dependent vasomotion in aging, hypertension, and heart failure. *Circulation* **87**, suppl. VII, 97–103.

Milner, P., Bodin, P., Loesch, A. & Burnstock, G. (1992). Increased shear stress leads to differential release of endothelin and ATP from isolated endothelial cells from 4- and 12-month-old male rabbit aorta. *Journal of Vascular Research* **29**, 420–425.

Mohri, K., Takeuchi, K., Shinozuka, K., Bjur, R. A. & Westfall, D. P. (1993). Simultaneous determination of nerve-induced adenine nucleotides and nucleosides released from rabbit pulmonary artery. *Analytical Biochemistry* **210**, 262–267.

Moncada, S., Palmer, R. M. J. & Higgs, E. A. (1991). Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacological Reviews* **43**, 109–142.

Moritoki, H., Matsugi, T., Takase, H., Ueda, H. & Tanioka, A. (1990). Evidence for the involvement of cyclic GMP in adenosine-induced, age-dependent vasodilatation. *British Journal of Pharmacology* **100**, 569–575.

Oshita, M., Kigoshi, S. & Muramatsu, I. (1993). Pharmacological characterization of two distinct α_1-adrenoceptor subtypes in rabbit thoracic aorta. *British Journal of Pharmacology* **108**, 1071–1076.

Pearson, J. D. & Gordon, J. L. (1979). Vascular endothelial and smooth muscle cells in culture selectively release adenine nucleotides. *Nature* **281**, 384–386.

Rubanyi, G. M. (1993). The role of endothelium in cardiovascular homeostasis and diseases. *Journal of Cardiovascular Pharmacology* **22**, suppl. 4, S1–14.

Sedaa, K. O., Bjur, R. A., Shinozuka, K. & Westfall, D. P. (1990). Nerve and drug-induced release of adenine nucleosides and nucleotides from rabbit aorta. *Journal of Pharmacology and Experimental Therapeutics* **252**, 1060–1067.

Shimokawa, H. & Vanhoutte, P. M. (1989). Dietary ω3 fatty acids and endothelium dependent relaxations in porcine coronary arteries. *American Journal of Physiology* **256**, H968–973.

Shinozuka, K., Bjur, R. A. & Westfall, D. P. (1988). Characterization of prejunctional purinoceptors on adrenergic nerves of the rat caudal artery. *Naunyn–Schmiedeberg's Archives of Pharmacology* **338**, 221–227.

Shinozuka, K., Hashimoto, M., Masumura, R. A., Bjur, R. A., Westfall, D. P. & Hattori, K. (1994*a*). In vitro studies of release of adenine nucleotides and adenosine from rat vascular endothelium in response to α_1-adrenoceptor stimulation. *British Journal of Pharmacology* **113**, 1203–1208.

Shinozuka, K., Kitagawa, S., Kunitomo, M., Yamaguchi, Y., Tanabe, Y., Fujiwara, M. & Hattori, K. (1994*b*). Release of endogenous ATP from the caudal artery in rats with arteriosclerosis. *European Journal of Pharmacology* **292**, 115–118.

Shinozuka, K., Kunitomo, M., Hattori, K., Bjur, R. A. & Westfall, D. P. (1995). Differences in purinoceptor modulation of norepinephrine release between caudal arteries of normotensive and hypertensive rats. *Journal of Pharmacology and Experimental Therapeutics* **272**, 1193–1198.

Shinozuka, K., Sedaa, K. O., Bjur, R. A. & Westfall, D. P. (1991). Participation by purines in the modulation of norepinephrine release by methoxamine. *European Journal of Pharmacology* **192**, 431–434.

Slakey, L. L. & Gordon, E. L. (1990). A comparison of ectonucleotidase activities on vascular endothelial and smooth muscle cells. (In *Biological Actions of Extracellular ATP*, ed. Dubyak, G. R. & Fedan, J. S.). *Annals of the New York Academy of Sciences* **603**, 366–379.

Tummino, P. J. & Gafni, A. (1991). A comparative study of succinate-supported respiration and ATP/ADP translocation in liver mitochondria from adult and old rats. *Mechanisms of Ageing and Development* **59**, 177–188.

Uchida, K., Nomura, U., Kadowaki, M., Takase, H., Tanao, K. & Takeuchi, N. (1978). Age-related changes in cholesterol and bile acid metabolism in rats. *Journal of Lipid Research* **19**, 544–552.

Westfall, D. P., Sedaa, K. O. & Bjur, R. A. (1987). Release of endogenous ATP from rat caudal artery. *Blood Vessels* **24**, 125–127.

Westfall, D. P., Sedaa, K. O., Shinozuka, K., Bjur, R. A. & Buxton, I. L. O. (1990). ATP as a cotransmitter. (In *Biological actions of extracellular ATP*, ed. Dubyak, G. R. & Fedan, J. S.). *Annals of the New York Academy of Sciences* **603**, 300–310.

Yamaguchi, M., Ozaki, K. & Suketa, Y. (1990). Alteration of glucose consumption and adenosine triphosphate content in bone tissue of rats with different ages: the stimulatory effect of zinc. *Chemical and Pharmaceutical Bulletin* **38**, 1660–1662.

Yang, S. Y., Cheek, D. J., Westfall, D. P. & Buxton, I. L. O. (1994). Purinergic axis in cardiac blood vessels: Agonist-mediated release of ATP from cardiac endothelial cells. *Circulation Research* **74**, 401–407.

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