Age-Related Changes in P2-Purinergic Receptors on Vascular Smooth Muscle and Endothelium

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The present study examined age-related changes in the vascular relaxation response to adenine nucleotides in hypertensive and normotensive rats. Aortic ring segments from normotensive Wistar-Kyoto (WKY) rats and spontaneously hypertensive rats (SHR), aged 4-6, 9-10, and 13-14 weeks, were examined for relaxation to adenosine 5'-triphosphate (ATP). The extent of ATP-induced relaxation in aortic ring segments with intact endothelium was unchanged with advancing age. Rubbed (endothelium-denuded) ring preparations at the age of 4-6 weeks showed a dose-dependent relaxation similar to that of the unrubbed rings. With advancing age, the ATP-induced relaxation in the rubbed rings decreased and was abolished. The relaxation response did not differ between the SHR and WKY animals at any age, whether the preparations were rubbed or unrubbed. The stable ATP analogue β,γ-methylene ATP induced a relaxation response similar to ATP in rubbed rings at 4-6 weeks of age. In addition, treatment with 8-phenyltheophylline did not diminish the relaxation induced by ATP. ATP-induced relaxation may be manifested via the direct action on the vascular smooth muscle in young rats and may be altered through the response mediated by endothelium with advancing age. This suggests that the vascular smooth muscle of young rats has a P2-purinergic receptor leading to relaxation and that this receptor activity declines with advancing age. In contrast, a P2-purinergic receptor leading to the generation of endothelium-derived relaxing factor may be activated in endothelial cells with advancing age. The alterations with age of P2-purinergic receptor in the vascular smooth muscle and endothelial cell are not affected by genetic hypertension. (Hypertension 1992;19:286-289)

KEY WORDS • adenosine triphosphate • age factors • purinergic receptors • vascular smooth muscle • endothelium

Adenosine 5'-triphosphate (ATP) may play an important dual role in the regulation of vascular tone: 1) as a vasoconstrictor acting via the excitatory P2-purinergic receptors (P2x-purinergic receptor) located on vascular smooth muscle and 2) as a vasodilator acting via the inhibitory P2-purinergic receptors (P2y-purinergic receptor) located on vascular endothelial cells to release an endothelium-derived relaxing factor (EDRF). Thus, a variety of studies in isolated preparations demonstrate that the relaxation of the vasculature by ATP requires the presence of intact endothelium, although there are few reports of an endothelium-independent vasodilation by ATP. Burnstock and his group demonstrated the presence of inhibitory and excitatory receptors (P2y and P2x purinergic receptors, respectively) in smooth muscle in rabbit portal vein and rabbit mesenteric and hepatic arteries. However, no studies have previously dealt with age- and hypertension-related alterations in the ATP-induced vascular relaxant responses. Therefore, in the current study, we examined the relaxant response to ATP in aorta with the endothelium intact or removed by rubbing from spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto (WKY) rats at different ages.

Methods

Age-matched male rats, SHR and WKY, aged 4-6, 9-10, and 13-14 weeks, were maintained in the Institute of Experimental Animals in Kyushu University. They were fed a commercial diet containing approximately 250 mg sodium and 800 mg potassium per 100 g. The average age and weight of SHR and WKY rats in each group were: 5.0±0.3 weeks, 86±12 g (n=13) and 4.7±0.3 weeks, 93±15 g (n=21) at 4-6 weeks of age; 9.4±0.3 weeks, 240±8 g (n=9) and 9.3±0.2 weeks, 244±6 g (n=9) at 9-10 weeks of age; 13.5±0.2 weeks, 295±3 g (n=8) and 13.4±0.2 weeks, 302±4 g (n=7) at 13-14 weeks of age, respectively.

After measuring the systolic blood pressure with tail-cuff plethysmography in the unanesthetized state, the animals were stunned and bled. The thoracic descending aorta was immediately excised and placed at room temperature in a modified Krebs solution of the following composition (mM): Na+ 137.4, K+ 5.9, Mg2+ 1.2, Ca2+ 2.5, Cl- 134.4, H2PO4− 1.2, HCO3− 15.5, and glucose 11.5. The solution was aerated with 95% O2-5% CO2, and the pH was maintained at 7.2-7.3. The aortas were cleaned of adherent connective tissue and cut into rings (5 mm long) under a dissection microscope. In some preparations, the endothelium was removed by gently rubbing the intimal surface with poly-
ethylenetubing. The remaining aortic rings were carefully handled so as not to injure their inner surfaces. The rings were mounted in a 25 ml volume organ bath, and perfusion was carried out at a rate of 3 ml/min (Micro tube pump MP-32, Tokyo Rakikai Co. Ltd., Tokyo) at a temperature of 35°C–36°C using a thermostatic bath with a pump (H-80, Taiyo Co. Ltd., Tokyo). Two stainless steel wires were inserted through the lumen of the aortic ring; one was anchored to a stationary support and the other was connected to a strain gauge transducer (UL-10GR, Shinkoh Co. Ltd., Nagano, Japan) coupled to a polygraph. The optimal resting tension in the ring preparations in the three different age groups was adjusted to 1.0 g at 4-6 weeks of age; 2.5 g at 9-10 weeks of age; and 3.0 g at 13-14 weeks of age, respectively. Tissues were allowed to equilibrate for at least 60 minutes.

In preliminary experiments, dose–response relations to norepinephrine (10^-9 to 10^-5 M) were constructed. The dose of norepinephrine producing 60–70% of maximal contractile response was estimated to be 3x10^-7 M. ATP (10^-7 to 3x10^-5 M) was applied in a cumulative fashion to aortas preconstricted by norepinephrine (3x10^-7 M). In addition, in the norepinephrine-induced preconstricted aortic ring segments without endothelium from 4-6-week-old rats, we examined the effects of 8-phenyltheophylline (3x10^-6 M), a potent antagonist at P<sub>r</sub>-purinergic receptor, on the relaxation response to ATP. Further, we examined a concentration–response curve to the metabolically stable ATP analogue 8-methylene ATP (10^-7 to 3x10^-5 M). Relaxation was expressed as a percentage of the contractile response to norepinephrine (3x10^-7 M).

The functional denudation of endothelium was confirmed by demonstrating a complete absence of relaxation induced by acetylcholine (10^-3 M) in the rubbed preparations. In addition, after completion of the experiments, all rings were fixed in half-Kalnovsky fixatives, and the presence or absence of endothelial cells was verified by scanning electron microscopy.

ATP, 8-methylene ATP, adenosine, and acetylcholine chloride were all obtained from Sigma Chemical Co., St. Louis, Mo. They were dissolved in distilled water. Norepinephrine hydrochloride (Sigma) was dissolved in distilled water containing 100 µM ascorbic acid. The agent 8-phenyltheophylline (Sigma) was dissolved in 80% vol/vol methanol containing 0.2 M NaOH. Nω-Nitro-L-arginine (Sigma) was dissolved in saline (0.9% wt/vol NaCl) acidified to pH 4.0 with 4N HCl. Drug concentrations are expressed as final molar concentrations in the bath solutions.

Results

The systolic blood pressures of SHR and WKY rats in the three different age groups were: 114±2 mm Hg and 105±7 at 4-6 weeks, 178±6 and 131±3 at 9-10 weeks, and 212±5 and 154±3 at 13-14 weeks, respectively. The systolic blood pressures of SHR at 9-10 and 13-14 weeks of age were significantly higher than those of the corresponding WKY rats (p<0.001).

Dose–response curves to ATP for the three different age groups appear in Figure 1. In all preparations with intact endothelium, ATP was effective in evoking dose-dependent relaxation responses that were essentially identical among age groups tested for both SHR and WKY rats. In rubbed preparations from rats aged 4-6 and 9-10 weeks showed a significant dose-dependent relaxation to ATP, whereas the degree of relaxation response was decreased at 9-10 weeks as compared with 4-6 weeks (SHR, p<0.05; WKY, p<0.05). Further-

![Figure 1. Line graphs show dose-response curves for vascular relaxation to adenosine 5'-triphosphate (ATP) in spontaneously hypertensive rats (SHR) (upper panels) and Wistar-Kyoto (WKY) rats (lower panels) of three different age groups. Relaxation in unrubbed (endothelium intact) and rubbed (endothelium removed) aortic rings to the indicated doses of ATP are shown. Relaxation was expressed as a percentage of the contractile response to norepinephrine (3x10^-7 M).](http://hyper.ahajournals.org/)

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The present study demonstrated that ATP causes a dose-dependent relaxation response not only in all unrubbed aortic ring preparations but also in the rubbed preparations in rats aged 4–6 and 9–10 weeks old. Although the relaxation response in the unrubbed rings was not altered by age in either the SHR or WKY rats, the rubbed ring preparations displayed less relaxation to ATP with advancing age. Ring segments from animals 4–6 weeks old displayed a similar relaxation response to ATP between the rubbed and unrubbed preparations in both SHR and WKY rats. Moreover, \( N^\text{G} \)-nitro l-arginine, a potent inhibitor of EDRF generation, did not alter ATP-induced relaxations in the unrubbed rings from rats 4–6 weeks old. These results imply that ATP-induced relaxation response in young rat aorta is principally endothelium-independent.

Previous investigators have reported that the ATP-induced relaxation in vessels of several species, including rat aorta, is an endothelium-dependent response mediated by EDRF.\(^{3-5,15}\) However, it has been noted that ATP-induced vasodilatation is not always mediated by the endothelium and that in some blood vessels, the response may be independent of the endothelium or a mixture of both endothelium-dependent and endothelium-independent mechanisms.\(^{5-9}\) Our study demonstrates that the mechanism of relaxation to ATP in rat aorta is altered dramatically with age. Although the ATP-induced relaxation was virtually endothelium-independent at 4–6 weeks of age, it became completely endothelium-dependent by 13–14 weeks of age. In previous studies, the relaxation response of the rat aorta to purine nucleotides was examined in adult animals.\(^{13,15,16}\) Therefore, it was suggested that those relaxation responses were always endothelium-dependent. In contrast, as in the present study, age-related changes in the response of isolated rat arteries to vasodilator agents have been reported with numerous drugs such as isoproterenol,\(^ {17,18}\) acetylcholine,\(^ {19,20}\) and histamine.\(^ {21}\)

The mechanism for ATP-induced endothelium-independent relaxation in the aorta of young rats could in principle be due to any of the following: 1) a \( P_2 \)-purinergic receptor in vascular smooth muscle after rapid breakdown to adenosine;\(^ {22} \) 2) in young rats, ATP acts directly via a \( P_2 \)-purinergic receptor in vascular smooth muscle as well as adenosine; 3) there is a \( P_2 \)-purinergic receptor responsible for relaxation \( (P_{2Y}-\text{purinergic receptor}) \) on the vascular smooth muscle of young rats. Our finding that even in the presence of 8-phenyltheophylline, a potent \( P_2 \)-purinergic receptor antagonist, the ATP-induced relaxation in rubbed rings is not abolished excludes the possibility that ATP acts via a \( P_2 \)-purinergic receptor either directly or after breakdown to adenosine. Moreover, the occurrence of relaxation responses to the metabolically stable analogue \( \beta \)-methylene ATP in rubbed rings from 4–6-week-old rats indicates there may be a \( P_2 \)-purinergic receptor leading to vasodilation \( (P_{2Y}-\text{purinergic receptor}) \) on the smooth muscle in the aorta of young rats.

In previous studies, it was proposed that the smooth muscle of the rabbit portal vein and mesenteric artery have inhibitory and excitatory \( (P_{2Y} \) and \( P_X \), respectively) purinergic receptors,\(^ {23,24} \) but the other vascular smooth muscles, including rat aorta, have only \( P_2 \)-purinergic receptors leading to contraction \( (P_X-\text{purinergic receptors}) \).\(^ {14} \) We are the first to demonstrate that the smooth muscle of young rat aorta also has a \( P_2 \)-purinergic receptor leading to relaxation \( (P_{2Y}-\text{purinergic receptors}) \).
ergic receptor). Furthermore, we suggest that the P2-purinergic receptor activity responsible for relaxation (P2Y-purinergic receptor) may decrease with increasing age as seen with β-receptor activity in the smooth muscle of rat aorta.17,18

In the present study, the ATP-induced relaxation of rat aorta was converted from being endothelium-independent in younger animals to endothelium-dependent with advancing age. This indicates that the ATP-induced relaxation of the aorta of 4–6-week-old rats rarely involves EDRF, whereas that effect in older rats involves EDRF. We previously observed that acetylcholine induced an endothelium-dependent relaxation of the aorta in 4–6-week-old rats.15,20 This suggests that the attenuation of EDRF action by the stimulatory effect of ATP on the endothelium of those younger rats involves changes in either density of the P2-purinergic receptors or a receptor-coupling mechanism within the endothelial cell, rather than the generation of EDRF, or the response of smooth muscle to EDRF. We previously demonstrated that the extent of the ATP-induced endothelium-dependent relaxation did not change in rats aged 3–6 months and 12–25 months.19,20 Therefore, in the present study, the occurrence of ATP-induced endothelium-dependent relaxation with increasing age suggests a consequence of growth and development rather than of old age.

Numerous investigators have reported that both endothelium-dependent and endothelium-independent relaxation are decreased in hypertension.23–26 However, ATP-induced relaxation, whether in rubbed or unrubbed preparations, was not affected by genetic hypertension in this study. This finding suggests that age-related changes in P2-purinergic receptors may not be involved in the initiation and maintenance of hypertension.

We conclude that with maturity, ATP-induced relaxation in the rat aorta is converted from being endothelium-independent to endothelium-dependent, suggesting that a P2-purinergic receptor responsible for relaxation (P2Y-purinergic receptor) on the vascular smooth muscle is activated at a young age and, with advancing age, its receptor activity declines. Alternatively, a P2-purinergic receptor leading to the generation of EDRF (P2Y-purinergic receptor) on the endothelial cell is activated. In hypertension, the ATP-induced relaxation response was not altered at any age.

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References

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