Participation of Endogenous Noradrenaline in the Release of ATP by High Potassium in the Rat Caudal Artery

Kazumasa Shinozuka¹, Masaru Kunitomo¹, Keiko Shimoura² and Keisuke Hattori²

¹Department of Pharmacology, Faculty of Pharmaceutical Science, Mukogawa Women’s University, 11-58, Koshien Kyuban-cho, Nishinomiya 663, Japan
²Department of Pharmacology, Shimane Medical University, Izumo 693, Japan

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ABSTRACT—Potassium (40 mM) evoked the release of adenine nucleotides and adenosine in rat isolated caudal arteries. This release was abolished by bunazosin. Endogenous noradrenaline was also released. Furthermore, the high potassium-induced purine release was abolished by rubbing the lumen of the vessel. These findings suggest that the high potassium releases endogenous noradrenaline, which subsequently elicits the release of ATP and related purines by acting on α₁-adrenoceptors on the endothelial cell.

Keywords: ATP release, Noradrenaline, Endothelium

Adenine nucleotides and adenosine are generally considered to be released upon nerve stimulation of a variety of sympathetically innervated tissues. In the caudal arteries of rats, electrical nerve stimulation and an α₁-adrenoceptor agonist, methoxamine, have been shown to evoke the release of a large amount of adenine nucleotides and adenosine from extraneuronal sites (1, 2). Sedaa et al. (3) suggested that in rabbit aorta, the source for the adenine nucleotides and adenosine released by methoxamine is primarily the endothelial cells. However, the details of the ATP release from the endothelium have not been sufficiently identified. In the present study, we examined the effect of high potassium on the release of ATP from the rat caudal artery to characterize the release of ATP.

The caudal artery (generally about 9 cm) was removed from male Sprague Dawley rats (SLC, Hamamatsu; ranging from 15 to 20 weeks of age) anesthetized with sodium pentobarbital (50 mg/kg, i.p.). In the release experiments, the caudal artery was cut open lengthwise; and for the measurement of contractile activity, the artery was cut into small rings 2–3 mm in length. Some experiments were carried out with arterial preparations in which the endothelial cell lining was disrupted. This was accomplished by gently rubbing the lumen of the artery with a stainless steel wire. In the release experiment, the whole caudal arterial preparation was suspended in a water-jacketed organ chamber containing 2.0 ml of a modified Krebs solution and allowed to equilibrate for 60 min. The medium was replaced every 3 min in the latter half of the equilibration period. After the equilibration, the bathing solution was rapidly collected (0-min sample: basal overflow for 3 min) by draining the organ chamber. Then the tissue was stimulated for 6 min with 40 mM potassium or 1.0 μM norepinephrine, and the bathing solution was collected twice at 3-min intervals; i.e., 3-min and 6-min samples, respectively. The purines, such as ATP, ADP, AMP and adenosine, and noradrenaline in the sample solution were analyzed by high performance liquid chromatography with fluorescence detection (4) and electrochemical detection (5), respectively. To compare the contractions induced by potassium and noradrenaline, the ring preparations of the caudal artery from four rats were stimulated first with noradrenaline and then with potassium at 30-min intervals. Another four preparations were stimulated with potassium and then with noradrenaline. The values were expressed as a percentage of the maximum contraction induced in each artery by the 6-min treatment with 1.0 μM noradrenaline. The details of the experiments have been described previously (5). All drugs and reagents were obtained from commercial sources. The data are reported as the mean±S.E.M. of at least four individual experiments and were evaluated for statistical significance by the Duncan new multiple range test. The 0.05 level of probability was accepted to indicate significance.

In the caudal artery, 40 mM potassium significantly increased the overflow of ATP and AMP, but not that of ADP or adenosine in the 6-min sample (Fig. 1). The over-
flow of total adenyl purines, sum of ATP, ADP, AMP and adenosine in the 6-min sample, was also significantly increased by the potassium. When bunazosin (1.0 μM) was added 15 min prior to and during stimulation with the high potassium concentration, the basal overflow of total purine (0.758±0.118 pmol/mg, n=4) was not significantly increased by the potassium (0.811±0.054 pmol/mg, 3-min; 1.026±0.102 pmol/mg, 6-min). Also, in the rubbed preparations, there were no significant differences between the basal overflow of total purine (0.480±0.052 pmol/mg, n=5) and the potassium-induced overflows (0.561±0.097 pmol/mg, 3-min; 0.689±0.151 pmol/mg, 6-min).

The high potassium concentration evoked a significant release of endogenous noradrenaline in the rat caudal artery as shown in Fig. 2. Exogenously applied noradrenaline (1.0 μM) markedly increased the basal overflow of total purine (0.694±0.045 pmol/mg, n=6) to 6.394±0.531 pmol/mg and 3.694±0.130 pmol/mg in the 3-min and 6-min samples, respectively. The amount of release of purines evoked by noradrenaline was markedly higher than that evoked by the high potassium concentration. Such a release of purines by noradrenaline was abolished by bunazosin (5). In the ring preparation of the caudal artery, a 6-min treatment with a high potassium concentration produced a marked contractile response that was larger than response produced by 1.0 μM noradrenaline (Fig. 3). The high potassium-induced contraction was not affected by bunazosin at 1.0 μM.

The major finding of the present study was that the high potassium-evoked purine release was inhibited by α1-adrenoceptor antagonists in the rat caudal artery. This indicates that the endogenous noradrenaline released from the sympathetic nerves by depolarization caused by the high potassium concentration stimulated the α1-adrenoceptors, which elicits the release of adenine nucleotides and adenosine. Katsuragi et al. (6) have suggested postjunctival ATP release from vas deferens of guinea pig is elicited by stimulation of α1-adrenoceptors.

In the present study, we observed that the release of adenine nucleotides and adenosine by potassium of high concentration was abolished by rubbing the lumen of the
rat caudal artery. Accordingly, a major source of adenyl purine released by the high potassium seems to be the endothelium. Recently, this idea has been directly demonstrated by the results of studies with cultured cardiac (7) and vascular (5) endothelial cells. Furthermore, most of ATP released from the endothelial cells seems to be rapidly metabolized to adenosine by ectonucleotidase (5).

Mechanical and physical stimulations, such as flow shear stress or stretching, have been reported to evoke the release of EDRF, endothelium derived relaxing factor, endothelin and ATP from endothelial cells (8–10). This leads to the possibility that the contraction induced by the high potassium as a mechanical or physical stimulation participates in the purines release. However, the present study shows that: 1) an α1-adrenoceptor antagonist, bunazosin, depressed the potassium-induced release of purines but did not affect the potassium-induced contraction and 2) there was no relationship between the amount of purines released and the amplitude of the contraction induced by two stimulants, the high potassium and noradrenaline. Therefore, the release of purines by the high potassium concentration seems not to be associated with mechanical contraction. Our recent observation (5) that the α2-adrenoceptor agonist clonidine causes vasoconstriction in the rat caudal artery but does not evoke a significant release of purines also supports this notion.

Taken together, we conclude that an α1-adrenoceptor-coupled mechanism for ATP release exists on the vascular endothelial cells. However, this notion raises the question of whether the endothelium can respond to adrenergic neurotransmission, because the distance for the diffusion of noradrenaline from adrenergic nerves located on the adventitial surface of the blood vessel to the endothelium is relatively large. Parker et al. (11) showed the nerve stimulation-induced efflux of 3H-noradrenaline from the intimal surface of the rabbit ear artery. Gonzalez et al. (12) also suggested that amine neurotransmitters including noradrenaline are able to interact in a very efficient manner with receptors on the endothelium. From these observations, it is considered that noradrenaline released from sympathetic nerves evokes the release of ATP via α1-adrenoceptors on vascular endothelial cells.

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