Endogenous ATP Released by Electrical Field Stimulation Causes Contraction via P2x- and P2y-Purinoceptors in the Isolated Tail Artery of Rats

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ABSTRACT—Electrical field stimulation (EFS) caused contraction of isolated tail arteries of rats. The EFS-induced contraction showed frequency-dependence and was entirely abolished by the sodium channel blocker tetrodotoxin (1×10^{-7} M). The EFS-induced (at 20 Hz) contraction was reduced by about 60% in the presence of phentolamine (1×10^{-6} M). Therefore, later experiments were carried out in the presence of phentolamine. Pyridoxal-phosphate-6-azophenyl-2',4'-disulphonic acid (PPADS) (1×10^{-8} – 1×10^{-6} M) and basilen blue E-3G (3×10^{-5} – 5×10^{-5} M), P2-receptor antagonists, significantly inhibited the contraction evoked by EFS. In addition, PPADS significantly inhibited the contractions induced by ATP (1×10^{-4} M) and a selective P2x-receptor agonist, α,β -methylene ATP (1×10^{-6} M). In contrast, basilen blue E-3G did not inhibit α,β -methylene ATP-induced contraction. The ecto-ATPase activator apyrase (5 and 10 U/ml) significantly reduced the EFS-induced contractions. These findings suggest that endogenous ATP released by EFS causes contractions of rat tail artery via both the P2x-receptors and P2y-receptors.

Keywords: P2x-receptor, P2y-receptor, Tail artery, ATP, Vasocontraction

ATP is a neurotransmitter or a co-transmitter in the central and peripheral nervous systems (1–3). ATP released as a co-transmitter with noradrenaline (NA) from the sympathetic nerves causes contraction of vascular smooth muscle via P2-receptors (4, 5). Recent functional studies of agonist potencies have led to classification of P2-receptors into two major types, P2x- and P2y-receptors (6, 7). Biochemical and cloning studies on P2-receptors have shown that the P2x-receptor is coupled with a ligand-gated ion channel (8–10) and that P2y- and P2u-receptors are coupled with G-proteins in the production of inositol 1,4,5-triphosphate (IP₃) (11, 12).

Recently, Bo and Burnstock (13) reported that smooth muscle of the rat tail artery has a high density of P2x₁-receptors. Moreover, Bao and Stjärne (14) reported that the ATP released by electrical field stimulation (EFS) exerts two opposite effects, excitatory and inhibitory effects, via “P2x-like” receptors in rat tail artery. Although there is much evidence for vasocontractile responses induced by exogenous application of ATP agonists (15, 16), little is known about which P2-receptor subtypes are involved

in the endogenous ATP-induced vasocontraction of arteries. Therefore, in the present study, we examined the mechanism of vasocontraction induced by endogenous ATP released by EFS in isolated tail arteries of rats and obtained evidence that endogenous ATP causes vasocontraction mediated mainly by P2x- and P2y-receptors.

MATERIALS AND METHODS

Preparations

Male Wistar rats (250–350 g) were killed by decapitation and their tail arteries were removed. The arteries were placed in chilled Krebs buffer solution (118.7 mM NaCl, 4.7 mM KCl, 1.8 mM CaCl₂, 1.2 mM KH₂PO₄, 1.2 mM MgSO₄, 24.8 mM NaHCO₃ and 10.1 mM glucose; pH 7.4). They were then freed from adipose and connective tissues under a microscope and cut into 3-mm-long rings. Each ring was mounted on an L-shaped wire attached to a force-displacement transducer (NEC-Sanei, Tokyo) in a 5-ml organ bath (US-5; UFER, Kyoto). The organ bath was maintained at 37°C and bubbled with a mixture of 95% O₂ and 5% CO₂.

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Vasoconstriction responses

Experiments on vasoconstrictile responses were performed as described previously (17). Briefly, the arterial rings were mounted in the organ bath and allowed to stabilize for 2 h. Then, the resting tension was adjusted to 0.3 g. The arterial rings were allowed to equilibrate for about 30 min in normal medium, which was replaced every 10 min. Before the experiments, equilibration was confirmed by 50 mM K^+ -pre-contraction responses. After the equilibration period, vasoconstrictile responses to drugs were measured. To remove the endothelium, the tail artery was connected with a syringe, and 5 ml of Krebs solution was injected rapidly 4–5 times. After each experiment, the presence of the endothelium was confirmed by demonstrating the abolition or marked suppression of relaxation produced by acetylcholine (ACh, 1×10^{-5} M).

EFS

EFS was applied through a pair of platinum electrodes (3.5-mm width) fixed on either site of the ring preparation of the rat tail artery (18). Stimulations were for 0.1-ms duration at 1–20 Hz, supramaximal voltage, for 10 s by use of an electronic stimulator (SEN-3301; Nihon Kohden, Tokyo). Frequency-response curves to EFS were performed with an interval of 1 min between each stimulation. A washout period of at least 1 h was used to avoid desensitization.

Responses are presented as percentages of the value of controls. Results are given as the mean $\pm$ S.E.M. Data were analyzed by Dunnett's *t*-test, $P < 0.05$ being considered to be statistically significant.

Drugs

ACh was obtained from Daiichi Pharmaceutical Co. (Tokyo); phentolamine from Ciba-Geigy (Basel, Switzerland); atropine from Merck (Darmstadt, Germany); tetrodotoxin, α, β -methylene ATP, ATP, basilen blue E-3G and apyrase (adenosine 5'-triphosphatase) from Sigma (St. Louis, MO, USA); suramin and PPADS (pyridoxal-phosphate-6-azophenyl-2',4'-disulphonic acid) from RBI (Natick, MA, USA).

RESULTS

Figure 1 shows typical recordings of contractile responses by EFS in isolated rat tail arteries. EFS caused frequency-dependent contraction (Fig. 1A). The EFS-induced (at 20 Hz) contraction was reduced by about 60% in the presence of phentolamine (1×10^{-6} M) (Fig. 1B). Therefore, later experiments were carried out in the presence of phentolamine (1×10^{-6} M). To avoid desensitization, the arteries were washed fully with the buffer

before the EFS experiments. The response to EFS was recovered 1 h after washing. The EFS-induced contraction was totally abolished by the sodium channel blocker tetrodotoxin (1×10^{-7} M) (Fig. 1C).

To clarify the involvement of the P2-receptor in vasoconstrictile responses, we first examined the effects of a non-selective P2-receptor antagonist, suramin, on the

Fig. 1. Typical recordings of contractile responses by EFS in rat tail arteries with intact endothelium. A: Arteries were contracted frequency-dependently by EFS. B: Effect of phentolamine (1×10^{-6} M) on the EFS-induced (at 20 Hz). C: Effect of tetrodotoxin (1×10^{-7} M) on the EFS-induced response (at 20 Hz).

EFS-induced contraction. Suramin inhibited the EFS-induced contraction in a concentration-dependent manner ($IC_{50}=4.76 \times 10^{-4}$ M) (Fig. 2A). Figure 2, B and C, show

Fig. 2. Effects of P2-receptor antagonists on EFS-induced contraction of rat tail arteries. A: Effects of the non-selective P2-receptor antagonist suramin on EFS-induced contraction of rat tail arteries. Values are means $\pm$ S.E.M. for 4–6 experiments. B, C: Effects of PPADS and basilen blue E-3G, P2 receptor antagonists, on the EFS-induced contraction in rat tail arteries. Values are means $\pm$ S.E.M. for 4–6 experiments. * $P < 0.05$, ** $P < 0.01$: significantly different from the control value.

the effects of PPADS and basilen blue E-3G, P2-receptor antagonists, on the EFS-induced contraction. Both PPADS (1×10^{-8} – 1×10^{-6} M) and basilen blue E-3G (3×10^{-5} – 5×10^{-5} M) significantly inhibited the contraction evoked by EFS (20 Hz). The maximum inhibitory effects of PPADS and basilen blue E-3G were shown at 1×10^{-6} M and 5×10^{-5} M, respectively (Fig. 2: A and B).

To determine the subtype of P2-receptors mediating contractions induced by the EFS, we first tested the effect of PPADS and basilen blue E-3G on the contraction induced by a selective P2X-receptor agonist, α, β -methylene ATP, and a non-selective P2-receptor agonist, ATP. Both α, β -methylene ATP and ATP caused transient contraction, which reached a peak within 1 min. The potency of the contractile response to α, β -methylene ATP was about 5- to 10-fold more than that of ATP (Fig. 3). PPADS significantly inhibited the contractions induced by α, β -methylene ATP (1×10^{-6} M) and ATP (1×10^{-4} M). The inhibitory effect of PPADS on α, β -methylene ATP-induced contraction was more than that on ATP-induced contraction (Fig. 4A). In contrast, basilen blue E-3G did not inhibit the contraction induced by the selective P2X-receptor agonist α, β -methylene ATP, whereas it inhibited ATP-induced contraction (Fig. 4B).

Next, to confirm the endogenous ATP release from nerve terminals by EFS, we examined the effects of the ecto-ATPase activator apyrase on the EFS-induced contraction in rat tail arteries. Apyrase (5 and 10 U/ml) significantly reduced the EFS-induced contractions (Fig. 5).

DISCUSSION

This study shows that the endogenous ATP released by EFS in isolated tail arteries of rats causes vasoconstriction mediated mainly by P2x-receptors and in part by the P2y-receptors. The EFS-induced contraction was frequency-dependent and tetrodotoxin-sensitive (Fig. 1), but

Fig. 3. Dose-response curves of contractile responses to α, β -methylene ATP (O) and ATP (●) in rat tail arteries.

Fig. 4. Effects of P2-receptor antagonists on α,β -methylene ATP- and ATP-induced contraction of rat tail arteries. A: Effects of PPADS on α,β -methylene ATP- and ATP-induced contractions of rat tail arteries. Values are means $\pm$ S.E.M. for 4–6 experiments. B: Effects of basilen blue E-3G on α,β -methylene ATP- and ATP-induced contraction in rat tail arteries. Values are means $\pm$ S.E.M. for 4–6 experiments. * $P < 0.05$, ** $P < 0.01$: significantly different from the control value.

Fig. 5. Effects of ecto-ATPase activator apyrase on EFS-induced contraction of rat tail arteries. Values are means $\pm$ S.E.M. for 4–6 experiments. ** $P < 0.01$: significantly different from the control value.

these contractions were not inhibited by denudation of the endothelial cells and not blocked by atropine (data not shown). Moreover, the contractile response induced by EFS was observed in the presence of phentolamine, and its contraction was inhibited by the non-selective P2-receptor antagonist suramin. These results suggest that the EFS stimulates the release of ATP from the nerves terminals, probably sympathetic nerve terminals. This idea seems to be supported by the findings that the ecto-ATPase activator apyrase attenuated the EFS-induced contraction (Fig. 5), since ATP is hydrolyzed to adenosine by ecto-ATPase (19). On the other hand, investigators reported that the ecto-ATPase inhibitor ARL67156 (20) potentiates contractions elicited by exogenous ATP or UTP (15, 21). Moreover, adenosine broken down from ATP by ecto-ATPase is reported to stimulate presynaptic adenosine A1-receptors and then inhibit the release of ATP and NA (22, 23). However, other neurotransmitters or neuromodulators besides ATP may be involv-

ed in the EFS-induced contraction, since the tachykinin NK-3 agonist senktide and neuropeptide Y also cause contractile responses in rat tail arteries (Y. Takano et al., unpublished data).

The present studies showed that P2-receptors exist in rat tail arteries, since the non-selective P2-receptor antagonist suramin inhibited the contraction evoked by EFS. Our results are consistent with the report of Bao and Stjärne (14). Characterization of the P2-receptor in rat tail arteries has also been reported (24), and McLaren et al. (15) proposed that two populations of P2-receptors are present in rat tail arteries. In addition, the present studies showed that the P2-receptor antagonists PPADS (16, 25, 26) and basilen blue E-3G (27) significantly inhibited the contractile response evoked by EFS. However, it is difficult to determine which subtype of P2-receptors causes the contraction, because selective P2-receptor antagonists have still not been developed. Although PPADS is thought to be a selective antagonist of P2x-receptors (16, 25, 26), it has also been reported that PPADS acts on P2-receptor subtypes other than the P2x-receptors depending on its dose and the tissue applied (25, 28). Therefore, to determine the selectivity of PPADS and basilen blue E-3G in this study, we examined the effects of PPADS and basilen blue E-3G on the contractile responses of rat arteries induced by either the selective P2x agonist α,β -methylene ATP or the nonselective P2 agonist ATP, because particularly α,β -methylene ATP is widely recognized as an agonist of P2x-receptors (7).

The contraction evoked by α,β -methylene ATP was inhibited significantly by PPADS, but not by basilen blue E-3G. On the other hand, the contraction evoked by ATP was prevented significantly by PPADS and basilen blue E-3G (Fig. 4). In addition, basilen blue E-3G at the concentrations of 3×10^{-5} M and 5×10^{-5} M used in this study was sufficient to inhibit the effects of P2y-receptors since basilen blue E-3G has been shown to produced a relatively specific blockade of P2y-receptors in a narrow concentration range; e.g., 1×10^{-5} M to 3×10^{-5} M, at most 5×10^{-5} M (27, 29, 30). In fact, Simonsen et al. determined that basilen blue E-3G (earlier named reactive blue 2) acts on the subtype of P2y-receptors (27). Therefore, the present results suggest that PPADS acts as a selective P2x-receptor antagonist, while basilen blue E-3G acts on subtypes other than P2x-receptors, probably P2y-receptors, in isolated rat tail arteries.

Taken together, these findings suggest that endogenous ATP released by EFS causes contractions of rat tail arteries via both P2x-receptors and P2y-receptors.

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