Short Communication

Comparison of Endothelium-Related Responses to Nucleotides of Dog and Monkey Cerebral Arteries

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Received November 11, 2009; Accepted January 7, 2010

Abstract. We compared mechanical responses to uridine-5'triphosphate (UTP) and 2-(methylthio)adenosine-5'diphosphate (2MeSADP) of cerebral arteries isolated from dogs and monkeys. In the dog, UTP induced endothelium-independent contraction, whereas 2MeSADP induced endothelium-dependent relaxation that was abolished by *N*^G-nitro-L-arginine (L-NA). In the monkey, both UTP and 2MeSADP induced endothelium-dependent relaxation. L-NA largely inhibited the UTP-induced relaxation whereas it partially inhibited the 2MeSADP-induced relaxation, and both remaining relaxations were abolished by charybdotoxin plus apamin. In conclusion, dog and monkey cerebral arteries respond differentially to UTP and similarly to 2MeSADP; however, involvement of endothelium-derived relaxing factor in the endothelium-dependent relaxation by 2MeSADP is quite different between the two species.

Keywords: cerebral artery, endothelium-dependent relaxation, nucleotide

Nucleotides play important roles in the control of cerebral circulation. Uridine-5'triphosphate (UTP) is rich in the brain and caused a potent and prolonged contraction in the cerebral artery; thus it has been suggested as one of the candidates to induce cerebral vasospasm (1, 2). Vasoactive properties of adenine nucleotides such as ATP and ADP have also been reported in the cerebral circulation (3, 4). In the present study, we compared the mechanical response to UTP, a pyrimidine agonist, and 2-(methylthio)adenosine-5'diphosphate (2MeSADP), a purine agonist, in isolated dog and monkey cerebral arteries and found contrasting results for these nucleotides between the species.

The Animal Care and Use Committee at Shiga University of Medical Sciences approved the use of monkey and dog arteries in the present study.

Japanese monkeys (*Macaca fuscata*) and Beagles of either sex, weighing 6–12 kg, were used. Animals anesthetized with both ketamine (15 mg/kg, i.m.) and sodium pentobarbital (20 mg/kg, i.v.) were killed by bleeding from the carotid arteries. The brain was rapidly removed, and cerebral arteries were isolated and cleaned.

The arteries were helically cut into strips with special care being taken to preserve the endothelium. In some strips, the endothelium was removed by gently rubbing the intimal surface. Each specimen was vertically fixed between hooks in a muscle bath containing modified Ringer-Locke solution of the following composition: 120 mM NaCl, 5.4 mM KCl, 2.2 mM CaCl₂, 1.0 mM MgCl₂, 25.0 mM NaHCO₃, and 5.6 mM dextrose. The bathing media were maintained at 37 ± 0.3°C, aerated with a mixture of 95% O₂ and 5% CO₂, and the pH of the solution was 7.36–7.43. Resting tension was adjusted to 1.5 g for dog artery or 1 g for monkey artery, which was optimal for inducing a maximal contraction.

Isometric contractions and relaxations of cerebral artery strips were recorded. Firstly, the contractile response to KCl (30 mM) was obtained, which was used as 100% contraction. Then, the strips were repeatedly washed with fresh medium and equilibrated. The strips were pretreated with indomethacin (10 μM) and partially contracted with prostaglandin (PG)F_{2α} in a concentration range of 0.2–1 μM. UTP (1 nM–10 μM) or 2MeSADP (1 nM–10 μM) was applied cumulatively to the bathing media to obtain the concentration–response curves of the strips. When examining the receptor subtype(s) or the mechanism of relaxation, the strips were pretreated with antagonist(s) or inhibitor(s) for approximately 30 min

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Published online in J-STAGE on February 18, 2010 (in advance)

doi: 10.1254/jphs.09316SC

before the concentration–response curve for an agonist was obtained. At the end of each series of experiments, papaverine (0.1 mM) was applied to attain the maximal relaxation, which was used as 100% relaxation. The integrity of vascular endothelium was examined by relaxant responses to substance P (10 nM) in dog cerebral arteries (5) or histamine (10 μ M) under treatment with famotidine (10 μ M) in monkey cerebral arteries (6).

The results shown in the text and figures are expressed as mean values $\pm$ S.E.M. Statistical analyses were made by Student's unpaired *t*-test for two groups and by Tukey's test after one-way analysis of variance for three or more groups. A value of $P < 0.05$ was considered to be statistically significant. The drugs used were *N*^G-nitro-L-arginine (L-NA), substance P, charybdotoxin, and apamin (Peptide Institute, Minoh); indomethacin, famotidine, UTP, 2MeSADP, MRS2179, sodium nitroprusside (Sigma, St. Louis, MO, USA); PGF_{2 α} (Pharmacia-Upjohn, Tokyo); histamine (Kanto Chemicals Co., Tokyo); ketamine (Sankyo, Tokyo); pentobarbital and papaverine hydrochloride (Dainippon-Sumitomo Pharma Co., Osaka).

In the dog cerebral artery with intact endothelium, addition of UTP produced a concentration-related contraction. Removal of the endothelium did not influence the UTP-induced contraction in the dog cerebral artery (Fig. 1A). On the other hand, UTP produced a concentration-related relaxation in the monkey cerebral artery. Removal of the endothelium abolished the UTP-induced relaxation in the monkey cerebral artery (Fig. 1C). Treat-

ment with an NO synthase inhibitor L-NA (10 μ M) largely inhibited the relaxation by UTP, but did not abolish it in the monkey cerebral artery (Fig. 2A). The remaining endothelium-dependent relaxation resistant to indomethacin and L-NA was significantly inhibited by a combination of charybdotoxin (100 nM) plus apamin (1 μ M), an intermediate- and a small-conductance calcium-activated K⁺-channel blocker, respectively (Fig. 2A). Treatment with MRS2179 (10 μ M), a selective P2Y₁-receptor antagonist, tended to inhibit the UTP-induced relaxation but this did not reach statistical significance in the monkey cerebral artery (Fig. 3A). Relaxation induced by 10 nM SNP was not significantly inhibited by MRS2179 ($45.2 \pm 13.2\%$ vs. control $52.0 \pm 7.0\%$, $n = 4$).

In the dog cerebral artery, 2MeSADP produced a concentration-related relaxation. Removal of the endothelium abolished the relaxation (Fig. 1B). Treatment with L-NA abolished the relaxation by 2MeSADP in the dog cerebral artery (Fig. 2B). Similarly, 2MeSADP produced an endothelium-dependent relaxation in the monkey cerebral artery (Fig. 1D). Treatment with L-NA partially inhibited the relaxation by 2MeSADP (Fig. 2C). The remaining endothelium-dependent relaxation resistant to indomethacin and L-NA was abolished by treatment with a combination of charybdotoxin plus apamin (Fig. 2C). Treatment with MRS2179 potentially inhibited the relaxation by 2MeSADP (Fig. 3B).

Difference in the cerebrovascular reactivity to endogenous vasoactive substances has been reported among

Fig. 1. Concentration–response curves for UTP in dog (A) and monkey (C) and for 2MeSADP in dog (B) and monkey (D) cerebral arterial strips in the presence (E+, closed circle) and absence (E–, open circle) of the endothelium. Strips were treated with 10 μ M indomethacin and precontracted with PGF_{2 α} . Results are expressed as percentages of 30 mM KCl- or 0.1 mM papaverine-induced responses for contraction (A) or relaxation (B, C, and D), respectively. Vertical bars represent the S.E.M. of 8 experiments. * $P < 0.05$ vs. E+; ns, no significance.

Fig. 2. Concentration–response curves for UTP in monkey (A) and for 2MeSADP in dog (B) and monkey (C) cerebral arterial strips with intact endothelium in the absence (control, closed circle) and presence of 10 μ M L-NA (L-NA, closed triangle) or together with 100 nM charybdotoxin plus 1 μ M apamin (L-NA + ChTX + Ap, open triangle). Strips were treated with 10 μ M indomethacin and precontracted with PGF_{2 α} . Results are expressed as percentages of 0.1 mM papaverine-induced relaxation. Vertical bars represent the S.E.M. of 6–8 experiments. * P < 0.05 vs. control; # P < 0.05 vs. L-NA.

Fig. 3. Concentration–response curves for UTP (A) and 2MeSADP (B) in the absence (control, closed circle) and presence of MRS2179 (closed square) in monkey cerebral arterial strips. Strips were treated with 10 μ M indomethacin and precontracted with PGF_{2 α} . Results are expressed as percentages of 0.1 mM papaverine-induced relaxation. Vertical bars represent the S.E.M. of 3–5 experiments. * P < 0.05 vs. control.

species (7) and this can be explained by activation of different receptor subtypes, localizations, and/or signal transduction pathways in either the vascular smooth muscle or the endothelium. In particular, the process of endothelium-dependent relaxation depends on the relative contribution of the release of NO, PGI₂, or endothelium-derived hyperpolarizing factor (EDHF) (8).

In the present study, we found functional heterogeneity between monkey and dog cerebral arteries: firstly, different effects of UTP on the vascular tone and secondly, different contribution of endothelium-derived NO to the relaxation by 2MeSADP. In the dog cerebral artery, UTP induced solely contraction, which is consistent with reports from other species (1, 9–10). The contraction was neither inhibited nor potentiated by removal of the endothelium, indicating endothelial-independent contraction without modification by any endothelium-derived substance in this artery. The mechanism of UTP-induced

contraction in the cerebral artery was reported to be due to membrane depolarization and intracellular Ca²⁺ elevation that is mediated directly via activation of purinergic receptors on vascular smooth muscle (2, 11). In contrast, in the monkey cerebral artery, UTP caused relaxation that was abolished by removal of the endothelium. Similar endothelium-dependent relaxation by UTP was reported in human cerebral artery in a case study where endothelium-derived substance was not examined (12). In the bovine cerebral artery, endothelium-dependent relaxation by UTP was abolished by L-NA, suggesting the relaxation is dependent on NO (11). In the present study, however, the UTP-induced endothelium-dependent relaxation was mediated not only by NO but also by EDHF-like substances. Involvement of EDHF-like substance in the endothelium-dependent dilation by UTP was observed in the rat (13).

In the present study, since the UTP-induced relaxation

tended to be inhibited by MRS2179 but was statistically insignificant, the UTP-induced relaxation in the monkey cerebral artery therefore seems to be mediated via an endothelial purinergic receptor(s) probably other than the P2Y₁ receptor. Because of the complexity of P2 receptors, lack of selective antagonist(s), and the limited scope of this study, identification of the UTP-stimulated receptor subtype(s) responsible for the endothelium-dependent relaxation remains to be clarified. On the other hand, it is conceivable to conclude a functional absence of these endothelial P2Y receptors sensitive to UTP in the dog cerebral artery. Because of its known contractile property, UTP has been considered to be one of the candidates to induce cerebral vasospasm; however, this may not be the case for the primates. Therefore, advantages and limitations of this difference are better considered during extrapolation to humans of the results obtained from animal studies.

We previously reported that ATP and ADP, natural adenine nucleotides, induced relaxation in the dog cerebral artery (5) where the relaxations were attenuated by treatment with L-NA to the same extent as removal of the endothelium. Similarly in the present study, 2MeSADP, a synthetic and more selective purine agonist, mimicked the natural nucleotides in eliciting endothelium-dependent relaxation in the dog cerebral artery, and the relaxation was abolished by L-NA. Our studies indicate that possibility of nucleotide-stimulated endothelial mediators other than NO is, if any, minimal in the dog cerebral artery. Along with our results, ADP is reported to elicit cerebral dilations that are exclusively mediated by NO (3). On the other hand, the endothelium-dependent relaxation by 2MeSADP in the monkey cerebral artery was partially inhibited by L-NA, leaving a prominent EDHF component. Treatment with MRS2179 potentially blocked the relaxation by 2MeSADP. This indicates that the endothelial P2Y₁ subtype mainly mediates the relaxation by 2MeSADP. Both endothelial P2Y₁ and P2Y₂ receptors in peripheral arteries were reported to be coupled to the NO/cGMP cascade and are involved in the endothelium-dependent relaxation (14). It has been demonstrated that, differences in the mechanism of vasodilatation resulting from stimulation of endothelial P2Y₁ and P2Y₂ purinoceptors result from differential endothelial Ca²⁺ responses with separate thresholds for NO- and EDHF-dependent dilations (15). Therefore, the remaining relaxation induced by high concentrations of 2MeSADP under treatment with MRS2179 in the monkey cerebral artery may be mediated with P2Y₂ receptor, but this also remains to be clarified.

In conclusion, UTP elicits endothelium-dependent relaxation in monkey but not dog cerebral artery and the relaxation is mediated with NO. 2MeSADP elicits en-

dothelium-dependent relaxation in dog and monkey cerebral arteries, and the relaxation is mediated with solely NO in dog cerebral artery and with NO and EDHF-like substance in monkey cerebral artery.

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