Vasomotor Effects of Acetylcholine, Bradykinin, Noradrenaline, 5-Hydroxytryptamine, Histamine and Angiotensin II on the Mouse Basilar Artery

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ABSTRACT. We investigated the responsiveness of the mouse basilar artery to acetylcholine (ACh), bradykinin (BK), noradrenaline (NA), 5-hydroxytryptamine (5-HT), histamine (His) and angiotensin (Ang) II in order to characterize the related receptor subtypes in vitro. ACh and BK induced endothelium-dependent relaxation of precontracted arteries with U-46619 (a thromboxane A2 analogue). Atropine (a non-selective muscarinic receptor antagonist) and Nω-nitro-L-arginine (a NO synthase inhibitor, L-NNA) shifted the concentration-response curve for ACh to the right, whereas pirenzepine, methoctramine and pFHHSiD (muscarinic M1, M2 and M3 antagonists, respectively) had no significant effect. L-NNA and HOE140 (a B2 antagonist) shifted the concentration-response curve for BK to the right, whereas des-Arg9-[Leu8]-BK (a B1 antagonist) and indomethacin (a cyclooxygenase inhibitor) had no significant effect. NA failed to produce any vasomotor action. His and Ang II induced concentration-dependent contraction. Diphenhydramine (a H1 antagonist) shifted the concentration-response curve for His to the right, whereas cimetidine (a H2 antagonist) had no significant effect. Losartan (an AT1 antagonist) shifted the concentration-response curve for Ang II to the right, whereas PD123319 (an AT2 antagonist) had no significant effect. These results suggest that the H1 and AT1 receptor subtypes might play an important role in arterial contraction, whereas muscarinic receptor subtypes apart from M1, M2 and M3, and B2 receptors on the endothelium, might modify these contractions to relaxations.

KEYWORDS: cerebral artery, pA2, receptor subtype, vasoconstrictor, vasodilator

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Because of the very small internal diameter (0.09–0.14 mm) and length (4–5 mm) of the mouse basilar artery, little information has been available regarding its reactivity to intrinsic vasoactive substances, such as acetylcholine (ACh), bradykinin (BK), noradrenaline (NA), 5-hydroxytryptamine (5-HT), histamine (His) and angiotensin (Ang) II in vitro and the receptor subtypes involved.

The basilar artery runs along the ventral aspect of the medulla oblongata and supplies the brain with blood in reptiles [33], birds [20] and mammals. The responsiveness of this artery seems to reflect changes in cerebral blood flow and local microvascular pressure. Species differences in the responsiveness of this artery to intrinsic vasoactive substances have been reported, and some are very unique and characteristic. For example, NA, a well-known vasoconstrictor, induces contraction of the basilar artery in dogs [26], monkeys [24], guinea pigs [7] and rabbits [8], whereas it induces relaxation in that of cattle [3] and pigs [16]. The intensity of relaxation in pigs is much greater than that in cattle, and this larger relaxation induced by NA is one of the characteristics of porcine basilar artery. In other case, BK, which is a well-known vasorelaxing factor, induces relaxation in human basilar artery, but induces very strong contraction in equine basilar artery [25]. The contraction induced by BK is greater than that induced by NA, His or 5-HT, and this is also one of the characteristics of the equine basilar artery.

Differences in responsiveness to these vasoactive substances might be dependent on differences in the distribution of their receptor subtypes on smooth muscle or endothelial cells. To our knowledge, basilar arterial responsiveness to these vasoactive substances in one species of animal has never been similar to that of other species. Therefore, characterization of basilar arterial reactivity in different species of animal would appear to be useful for investigating evolutionary relationships among animals.

Mice are widely considered to be a prime model of inherited human disease and share 99% of their genes with humans [28]. They are the most commonly used vertebrate species, because of their availability, size, low cost, ease of handling and high reproduction rate. The routine availability of mouse models of various cerebral circulatory disorders like Alzheimer’s disease, migraine and stroke (4) requires characterization of the regulation of basal arterial tone. A study of basilar artery is important, because it is one of the major resistance vessels in the brain.
In the present study, therefore, we attempted to clarify in detail the responsiveness of isolated mouse basilar arteries to ACh, BK, NA, 5-HT, His and Ang II and the receptor subtypes involved.

MATERIALS AND METHODS

**Tissue preparation:** Adult male mice (ICR, weight: 40 ± 5 g, age: 4 months ± 15 days) were decapitated under diethyl ether anesthesia. The basilar arteries were then gently isolated from the brain and transferred to ice-cold physiological saline (119 mM NaCl, 4.7 mM KCl, 1.6 mM CaCl₂, 1.2 mM MgCl₂, 25 mM NaHCO₃, 1.2 mM KH₂PO₄ and 10 mM glucose), pH 7.4, aerated with carbogen (95% (v/v) O₂, 5% (v/v) CO₂). Each artery was immediately dissected free of adherent tissues under a stereomicroscope. All experiments were performed in accordance with the Kagoshima University Guidelines for Animal Experimentation.

**Reagents:** We used the following reagents and final concentrations: NA (10⁻⁹–10⁻⁵ M), His hydrochloride (10⁻⁶–10⁻³ M), diphenhydramine hydrochloride (10⁻⁷–10⁻⁴ M), cimetidine (10⁻⁵ M), Ang II acetate salt (10⁻⁹–10⁻⁵ M), losartan potassium (10⁻⁷ and 10⁻⁶ M), PD123319 di trifluoracetate salt (10⁻⁶ M), BK acetate salt (10⁻⁹–10⁻⁶ M), des-Arg⁹-[Leu⁸]-BK (10⁻⁵ M), methoctramine hydrate (10⁻⁶ M), No-nitro-L-arginine (L-NNA; 10⁻⁴ M) and sodium nitroprusside (SNP; 10⁻⁴ M); Sigma-Aldrich, St. Louis, MO, U.S.A.), 5-HT (serotonin)-creatine sulfate (10⁻⁹–10⁻⁵ M; Merck, Darmstadt, Germany), HOE140 (10⁻⁷ and 10⁻⁶ M; Peptide Institute, Osaka, Japan), indomethacin (10⁻⁵ M; Nacalai tesque, Kyoto, Japan), ACh chloride (10⁻⁹–10⁻⁵ M; Daichi Sankyo, Tokyo, Japan), pirenzepine dihydrochloride (10⁻⁶ M; Santa Cruz Biotechnology, Santa Cruz, CA, U.S.A.), hexahydro-sila-difenidol hydrochloride, p-fluoro analog (pFHHSiD, 10⁻⁵ M; Research Biochemicals, Natick, MA, U.S.A.) and U-46619 (10⁻⁷ M; Cayman Chemical Co., Ann Arbor, MI, U.S.A.). All drugs were dissolved in distilled water.

**Functional studies:** Two rings approximately 2 mm long were cut from each mouse basilar artery. Each ring was mounted horizontally between two L-shaped stainless steel holders (outer diameter 0.02 mm), with one part fixed to an isometric force transducer (TB-611T, Nihon Kohden Kogyo, Tokyo, Japan), and immersed in a 4-m l distilled water. Each suspended ring was left to equilibrate for at least 120 min under a resting tension of 0.03 g. This tension was chosen, because it allowed us to induce maximum contractions in the basilar artery. KCl (60 mM) was applied every 30 min until the amplitude of the contraction reached a constant value. Changes in the KCl concentration of the physiological saline were compensated for by equimolar adjustment of the NaCl concentration. The isometric tension was recorded with an amplifier (AP-621G, Nihon Kohden Kogyo, Tokyo, Japan), digitized with an analogue-digital converter (PowerLab/8SP, ADInstruments Co., Castle Hill, NSW, Australia) and stored on the hard disk of a personal computer. The cumulative concentration-response curve of each agonist was obtained by adding a solution of agonist directly to the fluid in the bath. Antagonists were added to the bathing media 30 min before the agonist. The antagonists had no effect on the resting vascular tone. The log concentration-ratio of EC₅₀ values (i.e., concentration producing half-maximum response) in the absence or presence of antagonist was calculated and plotted against the logarithm of antagonist concentration to obtain the pA₂ values [2].

**Statistical analysis:** Results are expressed as means ± SEM. Statistical analyses were performed by Student’s t-test or the Bonferroni test after one-way analysis of variance (Stat View J-4.5, Abacus Concepts Inc., Berkeley, CA, U.S.A.). Significance was established when the probability level was equal to or less than 0.5.

RESULTS

**Responsiveness to ACh, BK, NA, 5-HT, His and Ang II:** We generated concentration-response curves for ACh, BK, NA, 5-HT, His and Ang II using isolated mouse basilar arteries (Fig. 1). Contractile response was measured under the resting tone of normal artery, whereas the relaxation response was measured under contraction with U-46619 (a thromboxane A₂ analogue). ACh and BK induced relaxation in a concentration-dependent manner (Fig. 1B). NA did not induce any changes in the vascular tone. His and Ang II induced contraction in a concentration-dependent manner. 5-HT induced infrequent contraction (9 of 36 cases) (Fig. 1A).

**Responsiveness to L-NNA and indomethacin:** L-NNA (a NO synthase inhibitor, 10⁻⁴ M) induced contraction (13.4 ± 1.8% to 60 mM KCl) under resting tension, and indomethacin (a cyclooxygenase inhibitor, 10⁻⁵ M) induced relaxation (5.8 ± 0.5% to 10⁻⁴ M SNP) under contraction with L-NNA.

**Maximal responses and pEC₅₀ values for ACh, BK, NA, 5-HT, His and Ang II:** Table 1 shows the maximal responses and pEC₅₀ values for the agonists examined. BK was the most sensitive relaxing agent (pEC₅₀=6.84 ± 0.09) and induced the most potent maximum relaxation (−65.5 ± 4.3%), whereas Ang II was the most sensitive contracting agent (pEC₅₀=6.81 ± 0.08) and induced the most potent maximum contraction (57.9 ± 4.7%) of the mouse basilar artery.

**Effect of endothelial denudation, L-NNA, atropine, pirenzepine, methoctramine and pFHHSiD on ACh-induced relaxation:** We investigated the effects of endothelial denudation, L-NNA, atropine, pirenzepine (a M₁ receptor antagonist), methoctramine (a M₂ receptor antagonist) and pFHHSiD (a M₃ receptor antagonist) on the concentration-response curve for ACh. Atropine at 10⁻⁷ M and 10⁻⁶ M shifted the concentration-response curve for ACh to the right and at 10⁻⁷ M largely abolished the ACh-induced relaxation (Fig. 2A). The calculated pA₂ value for atropine was 8.02 ± 0.06 and its slope was 0.86 ± 0.05, which was not significantly different from unity (Fig. 2C). Figure 2B shows the effects of endothelial denudation, L-NNA, pirenzepine, methoctramine and pFHHSiD on ACh-induced relaxation under the contraction induced by U-46619. ACh-induced relaxation
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was completely abolished in endothelial denudated artery and significantly inhibited by L-NNA. None of the three antagonists had any significant effect on the ACh-induced relaxation.

Effects of endothelial denudation, L-NNA, indomethacin, and B1 and B2 receptor antagonists on BK-induced relaxation: Endothelial denudation had completely abolished BK-induced relaxation, and the NO synthase inhibitor L-NNA partially inhibits it. The cyclooxygenase inhibitor indomethacin had no significant effect on BK-induced relaxation (Fig. 3A). To characterize the BK receptor subtypes, the arteries were pretreated with B1 and B2 receptor antagonists. Figure 3B shows the effect of des-Arg9-[Leu8]-BK (a B1 receptor antagonist) on BK-induced relaxation of the mouse basilar artery. Des-Arg9-[Leu8]-BK (10−5 M) did not significantly affect BK-induced relaxation. This figure also shows the effect of HOE140 (a B2 receptor antagonist) on BK-induced relaxation. HOE140 shifted the BK-induced concentration-response curve to the right. The calculated pA2 value for HOE140 was 7.53 ± 0.12 and its slope was 1.03 ± 0.14, which was not significantly different from unity (Fig. 3C).

Effect of 5-HT on isolated mouse basilar artery: 5-HT induced concentration-dependent contraction in 9 of 36 mouse basilar arteries; the second-time 5-HT response being significantly lower than the first (Fig. 4). Endothelial denudation or inhibition of NO synthase by L-NNA had no effect on this phenomenon (data not shown).

Effects of diphenhydramine and cimetidine on His-induced contraction: We investigated the effects of diphenhydramine

Table 1. pEC50 values and maximal responses to agonists

| Agonists     | pEC50     | Max (%) |
|--------------|-----------|---------|
| Bradykinin   | 6.84 ± 0.09 | -65.5 ± 4.3a |
| Acetylcholine| 6.76 ± 0.06 | -46.4 ± 4.4a |
| Angiotensin II| 6.81 ± 0.08 | 57.9 ± 4.7b |
| 5-Hydroxytryptamine | 6.64 ± 0.10 | 18.1 ± 2.3b |
| Histamine    | 4.58 ± 0.02 | 41.6 ± 2.4b |
| Noradrenaline| –         | No response |

a: Relaxation induced by 10−4 M SNP (0.011 ± 0.002 g) was taken as 100%, b: Contraction induced by 60 mM KCl (0.038 ± 0.004 g) was taken as 100%, Each point represents the mean ± SEM of 8–12 mice.
(a H1 receptor antagonist) and cimetidine (a H2 receptor antagonist) on the concentration-response curve for histamine (His). Diphenhydramine (10⁻⁷–10⁻⁴ M) shifted the concentration-response curve for His to the right in parallel; cimetidine (10⁻⁵ M) had no significant effect (Fig. 5A). The calculated pA₂ value for diphenhydramine was 6.62 ± 0.11 and its slope was 0.81 ± 0.19, which was not significantly different from unity (Fig. 5B).

Effects of losartan and PD123319 on Ang II-induced contraction: We examined the effects of losartan (an AT1 receptor antagonist) and PD123319 (an AT2 receptor antagonist) on the concentration-response curve for angiotensin (Ang) II (Fig. 6). Losartan (10⁻⁷ and 10⁻⁶ M) shifted the concentration-response curve for Ang II to the right in parallel. The calculated pA₂ value for losartan was 8.12 ± 0.10 and its slope was 0.79 ± 0.03, which was significantly different from unity. PD123319 had no significant effect.

DISCUSSION

To our knowledge, this is the first study to have demonstrated the responsiveness of the isolated mouse basilar artery to ACh, BK, NA, 5-HT, His and Ang II. Although some of these vasoactive substances have been investigated by pressure myograph system previously [4], the receptor subtypes have not yet been described.

ACh is an endogenous substance producing endothelium-dependent vasorelaxation via NO, prostacyclin and/or endothelium-derived hyperpolarizing factor (EDHF). In the present study, ACh-induced relaxation was completely abolished by endothelial denudation and significantly inhibited by L-NNA as shown in Fig. 2B. These results suggested that ACh induces endothelium-dependent and NO-mediated relaxation in mouse basilar artery. Three types of muscarinic receptors appear to be involved in the relaxation or contraction of arteries [5], but in the present study, atropine shifted the concentration-response curve for ACh to the right with a pA₂ value of 8.02 as shown in Fig. 2A. There is no report regarding the pA₂ value of atropine.
in ACh-induced relaxation on mouse artery or other tissues. But, our calculated pA2 value is similar to that reported for the rabbit aorta (8.14) [12], but lower than that reported for the rat mesenteric artery (9.78) [29]. The differences might be due to the differences in artery and species of animals studied. M1, M2 and M3 receptor antagonists had no significant effect. These results differed from those obtained using chicken basilar arteries, where atropine (non-selective muscarinic receptor antagonist) and pHHHSID (selective M3 receptor antagonist), but not pirenzepine (selective M1 receptor antagonist) and methoctramine (selective M2 receptor antagonist), shifted the concentration-response curve for ACh to the right [15]. Our results suggest that some other receptor subtypes apart from M1, M2 and M3 receptors might be responsible for ACh-induced relaxation. Molecular cloning studies have revealed the existence of five molecularly distinct Ach receptor subtypes (M1-M5) [6, 30]. Studies of the expression of the cloned M5 receptor gene in cultured mammalian cells have shown that the encoded receptor protein is functional and efficiently couples to G proteins of the Gq family, similarly to the M1 and M4 receptor subtypes [5, 14]. As no M5 antagonist is commercially available, we were unable to characterize the receptor, although a previous study observed that ACh-mediated dilation of cerebral arteries and microvessels was virtually abolished in M5 receptor-knockout mice [32].

BK induced relaxation of mouse basilar arteries precontracted with U-46619, and this effect was abolished in arteries after endothelial denudation as shown in Fig. 3A. This result was consistent with the previous findings by Rosenblum et al. [21], who used light-dye or laser-dye techniques to show that cerebral vasodilator responses to BK in vivo were abolished after injury to endothelial cells in mice. Pretreatment with L-NNA partially shifted the concentration-response curve for BK to the right, and indomethacin had no significant effect on it. These results suggest that BK-induced relaxation might be partly mediated by NO, but not by arachidonic acid metabolites. Some other EDHF might also be involved in BK-induced relaxation of mouse basilar artery. The dilative action of BK on small pial arteries is reportedly mediated by release of hydroxyl radicals in mouse [21] and cat [13]. In line with this, one previous study has verified that BK-induced relaxation of human forearm resistance vessels did not involve NO or a vasodilator prostandion, this effect being mediated by hyperpolarization of the vascular wall [11]. Thus, it seems that the vasorelaxing pathways involved in BK-induced relaxation vary depending on the vascular bed studied. In the present study, the relaxing effect of BK was significantly inhibited by HOE140, but not by the B1 receptor antagonist des-Arg2-[Leu5]-BK as shown in Fig. 3B. These data indicate that the dilative effect of BK on the mouse basilar artery is mediated by the B2 receptor, and not by the B1 receptor. B2 receptor-mediated responses are generally not observed under normal physiological conditions [27]. The calculated pA2 value of HOE140 is 7.53, which is identical to the value for the human isolated umbilical artery, i.e. 7.50 [1].

In the mouse basilar artery, NA had no effect on resting vascular tone. This result was similar to that obtained by a previous study [7], which showed that NA had no effect on the rat basilar artery.

In mouse basilar artery, 5-HT induced contraction with an intensity of 18.1%, whereas in most species of animal, the intensity ranges from 40% to 100% [34]. The frequency of 5-HT-induced contraction was 25% among all experimental cases (9 of 36), and furthermore second-time responses were significantly lower than the initial ones and absent in some cases. After first application, a possible desensitization or internalization and/or down regulation of the 5-HT receptor might interfere second-time responses. A similar phenomenon has been reported in rabbit middle cerebral artery [23]. Therefore, we were unable to carry out subsequent experiments to characterize the receptor subtypes involved in the 5-HT-induced contraction. This brings into question the usefulness of the mouse as a model for studies of migraine and stroke, conditions in which 5-HT is thought to play important roles [19, 31].

The H1 receptor antagonist diphenhydramine shifted the concentration-response curve for His to the right, whereas the H2 receptor antagonist cimetidine had no significant effect on the His-induced contraction as shown in Fig. 5A. These results suggest that activation of the H2 receptor induces contraction of the mouse basilar artery. Contraction of resting vascular tone in response to His has also been reported in guinea pigs [7], pigs [17], humans [22], horses and cattle [18]. The calculated pA2 value for diphenhydramine was 6.62, which is lower than the values reported for bovine (7.61) and porcine (7.77) basilar arteries [17, 18]. A further study is needed to clarify the differences in pA2 values among species.

The effects of Ang II occur via activation of two receptor subtypes, AT1 and AT2. The vasocontractile effects of Ang II are generally considered to result from activation of the AT1 receptor. The selective AT1 receptor antagonist losartan shifted the concentration-response curve for Ang II to the right, whereas PD123319 (an AT2 receptor antagonist) had no effect as shown in Fig. 6A. These results suggest that the Ang II-induced contraction in mouse basilar artery is mediated by activation of AT1 receptors. A previous study showed that vasoconstriction of the cerebral artery in response to Ang II was markedly reduced in genetic AT1A-deficient mouse [9]. The calculated pA2 value for losartan is 8.12, which is similar to that reported in canine mesenteric (8.15) and pulmonary (7.96) artery [10].

L-NNA induced contraction and indomethacin induced relaxation through inhibition of NO synthase and cyclooxygenase, respectively. These results suggest that the resting tone balance of the mouse basilar artery is also maintained by spontaneous release of NO and thromboxane A2.

Small rodents, such as mice and rats, are frequently used in preclinical cerebrovascular research, mice being particularly useful, because an increasing number of transgenic models are becoming available. Mice are often used as small animal models of brain ischemia, venous thrombosis or vasospasm, and Alzheimer’s disease. The majority of novel therapeutic approaches are tested in small animal models of human
disease, especially those involving mice, prior to clinical testing. A variety of murine models of cerebrovascular disease are available, from which a number of molecular and structural elements of cerebral disorders have been clarified.

In summary, we have investigated the responses of the mouse basilar artery to a number of pharmacological agents that are modulators of cerebrovascular circulation in both normal and pathophysiological states. We have demonstrated that the mouse basilar artery is responsive to ACh and BK with relaxation, and to 5-HT, His and Ang II with contraction, but is unresponsive to NA. These response characteristics are unique to the mouse basilar artery.

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