2-Methyl-5-HT-Induced Vasoconstrictions Mediated via $\alpha_1$-Adrenoceptors in Rabbit Common Carotid Arteries

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ABSTRACT—We investigated the effects of 2-methyl-5-HT and 1-phenylbiguanide on isolated and perfused rabbit common carotid arteries by a cannula insertion method. 1-Phenylbiguanide produced neither vasoconstriction nor dilation. On the other hand, 2-methyl-5-HT produced only a vasoconstriction, and the dose-response curve was shifted to the right in parallel by treatment with either ketanserin or bunazosin, although methysergide and granisetron had no antagonistic effect. Moreover, the vasoconstriction was not inhibited by guanethidine and imipramine. These data showed that 2-methyl-5-HT acts as an $\alpha_1$-adrenoceptor agonist but not as a 5-HT$_3$-receptor agonist in this vessel.

Keywords: 2-Methyl-5-HT, 1-Phenylbiguanide, $\alpha_1$-Adrenoceptor

5-HT$_3$ receptors mainly exist in the central nervous system (1), but they also have complex and heterogeneous effects on the cardiovascular system (2–7). 1-Phenylbiguanide, a potent 5-HT$_3$-receptor agonist, elicited a Bezold-Jarisch-like bradycardic reflex in conscious rabbits (6). Blauw et al. (2) reported that 5-HT induced a vasodilation in the human forearm artery mediated via 5-HT$_3$ receptors. Recently, Tadipatri et al. (3–5) reported that the contractile effects of 2-methyl-5-HT, a selective 5-HT$_3$ agonist, on the rabbit isolated renal artery were mediated by 5-HT$_1$-like receptors. The rabbit common carotid artery has dominant 5-HT$_2$ receptors different from the renal artery (3), and whether 5-HT$_3$ receptors exist remains yet unknown. There is a possibility that vascular responses to a 5-HT$_3$ agonist is mediated by 5-HT receptors in this artery.

Thus, in the present study, we investigated the mechanisms of action of 5-HT$_3$-receptor agonists, 2-methyl-5-HT and 1-phenylbiguanide, in isolated and perfused rabbit common carotid arteries by the cannula insertion method developed and modified by Hongo and Chiba (8) and Tsuji and Chiba (9).

Twelve male albino rabbits weighing 2.0–2.5 kg were anesthetized with sodium pentobarbital (30 mg/kg, i.v.). After treatment with sodium heparin (200 units/kg), the animals were killed by rapid exsanguination from the abdominal aorta. The common carotid artery was then carefully isolated and cleaned, and the side branches were tied with silk threads. Segments (2.0–3.0 cm) were carefully cannulated with a stainless steel needle with small side holes at a 5 mm distance from the distal blind end (16 gauge) and set up for perfusion (8, 9). Krebs-Henseleit solution, gassed with 95% O$_2$ and 5% CO$_2$ and maintained at 37°C, was perfused by a micro-tube pump (Tokyo Rikakikai Co., Ltd., Tokyo). A vasoconstriction was observed as an increase in perfusion pressure. The perfusion pressure was continuously measured with an electric manometer (Nihon Kohden TP-200P, Tokyo). The perfusate contained: 118 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl$_2$, 1.2 mM KH$_2$PO$_4$, 1.2 mM MgSO$_4$, 25 mM NaHCO$_3$ and 10 mM glucose.

Drugs used were 2-methyl-5-hydroxytryptamine maleate (2-methyl-5-HT) and 1-phenylbiguanide (Research Biochemicals, Natick, MA, USA); phenylephrine hydrochloride (PE; Kowa, Tokyo); bunazosin hydrochloride (Eisai, Tokyo); imipramine hydrochloride (Fujisawa, Osaka); ketanserin tartrate and guanethidine (Sigma, St. Louis, MO, USA); methysergide maleate (Kissei, Nagano); and granisetron hydrochloride (Smith Kline Beecham, Tokyo). The drug was dissolved in physiological saline. The drug solution was bolusly administered into the rubber tubing close to the cannula in a volume of 0.01–0.03 ml for a period of 4 sec.

Results are expressed as the mean±S.E.M. Statistical differences between two means and two curves were determined by Student’s t-test, and significance was accepted...
when P < 0.05.

When 1-phenylbiguanide up to the dose of 10⁻⁶ mol was injected into the cannulated common carotid artery of the rabbit, neither vasoconstriction nor vasodilation was induced (data not shown). On the other hand, 2-methyl-5-HT produced a vasoconstriction in a dose-dependent manner (Fig. 1). We did not observe any vasodilation with 2-methyl-5-HT in preconstricted preparations induced by 10⁻⁵ M phenylephrine (data not shown). Methysergide (10⁻⁶ M) (a 5-HT₁ and 5-HT₂ antagonist) did not inhibit the response to 2-methyl-5-HT, but the dose-response curve for 2-methyl-5-HT was significantly suppressed after the treatment with 10⁻⁸—10⁻⁶ M ketanserin (a 5-HT₂ antagonist) (Fig. 1). Moreover, the response was not influenced by 10⁻⁶ M granisetron, a selective 5-HT₃-receptor antagonist (10), although it was markedly inhibited by 10⁻⁶ M bunazosin, a selective α₁-adrenoceptor antagonist (Fig. 1).

5-HT accumulates in the adrenergic nerves and can act as one of the sympathetic vasoconstrictor transmitters in cerebral arteries (11, 12). We determined whether 2-methyl-5-HT was uptaken into sympathetic nerve terminals, which in turn caused a release of norepinephrine (Fig. 2). When 3 × 10⁻⁵ M guanethidine was continuously infused in the perfusion line for more than 60 min, the perfusion pressure was slightly increased (18±4 mmHg, when P < 0.05.

Fig. 1. Effects of methysergide (A, n=8), ketanserin (B, n=9), granisetron (C, n=8) and bunazosin (D, n=8) on 2-methyl-5-HT-induced vasoconstrictions in isolated and perfused rabbit common carotid arteries. The symbols represent the following: the control (○) and 10⁻⁴ M (●), 10⁻³ M (▲) and 10⁻² M (■) of each antagonist. Points represent the mean values and vertical bars represent ± S.E.M.

Fig. 2. Effects of guanethidine (upper panel, n=7) and imipramine (lower panel, n=6) on 2-methyl-5-HT-induced vasoconstrictions in isolated and perfused rabbit common carotid arteries. The symbols represent the following: the control (○) and 3 × 10⁻⁵ M of each adrenergic neuron and uptake blocking agent (●). Points represent the mean values and vertical bars represent ± S.E.M.
n=7). However, 3 x 10^{-5} M guanethidine never inhibited the response to 2-methyl-5-HT significantly. We confirmed the same result in 3 x 10^{-5} M imipramine-treated preparations.

5-HT3 receptors have some roles within the cardiovascular system in addition to the peripheral and central nervous systems (2, 6, 10). 2-Methyl-5-HT is commonly considered to be selective for 5-HT3 receptors (1, 13). In the present study, ketanserin potently antagonized the action evoked by 2-methyl-5-HT, whereas methysergide did not. On the other hand, the vasoconstriction was not influenced by granisetron, a selective 5-HT3-receptor antagonist (10), but the response was markedly inhibited by bunazosin, a potent α1-adrenoceptor antagonist. Since ketanserin has an α1-adrenoceptor antagonistic property (14), these data indicate that 2-methyl-5-HT-induced vasoconstrictions are mediated via α1-adrenoceptors. To examine whether 2-methyl-5-HT stimulates sympathetic nervous factors, we used guanethidine and imipramine, but neither drug modified the 2-methyl-5-HT-induced responses; Furthermore, they also had no effect on the responses to potassium chloride (data not shown).

Recently, Tadipatri and Saxena (4) and Tadipatri et al. (5) reported that contractile effects of 2-methyl-5-HT on the rabbit renal artery are mediated by 5-HT1-like receptors, and this substance could sometimes elicit functional responses in tissue containing 5-HT1-receptor subtypes. They also confirmed this conclusion by ligand binding studies. In rabbit blood vessels, the renal or ear artery possesses a 5-HT1-like receptor, but 5-HT acts as a 5-HT2-receptor agonist in other arteries (3). In the present experiments, the two 5-HT1-receptor agonists did not cause any vasodilation, whereas 2-methyl-5-HT-induced vasoconstrictions were not influenced by methysergide. Thus, the involvement of 5-HT1 receptors in the response to 2-methyl-5-HT can be ruled out by these preparations.

1-Phenylbiguanide had no vascular effect in this study. Zschauer et al. (7) also reported that this drug had no contractile effect on the rabbit ophthalmic artery. Therefore, the two 5-HT3 receptor agonists have different properties in the rabbit common carotid artery, and 2-methyl-5-HT mainly stimulates α1-adrenoceptors in this vessel. From these results and those in the literature, 2-methyl-5-HT may exert its action directly via α1-adrenoceptors in rabbit common carotid arteries.

REFERENCES


