Potentiating Effect of Methysergide on Norepinephrine-Induced Constriction of the Isolated Internal Carotid Artery of the Dog

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Abstract—The stainless steel cannula inserting method was used to examine the effects of methysergide on 5-hydroxytryptamine (5-HT)- and norepinephrine-induced vasoconstriction in the isolated internal carotid artery of the dog. 5-HT, at a dose of 0.3 μg, induced a marked increase in perfusion pressure, usually over 100 mm Hg. On the other hand, norepinephrine produced a relatively small increase in perfusion pressure (20–40 mm Hg) at a large dose of 10 μg. Methysergide inhibited 5-HT-induced vasoconstriction. Norepinephrine-induced vasoconstriction was significantly potentiated by treatment with methysergide and blocked by phentolamine. Methysergide also enhanced the vasoconstrictor response to potassium chloride. Thus, it is suggested that the potentiating effect of methysergide on norepinephrine-induced vasoconstriction may partially be due to activation of the inward calcium channel of the internal carotid artery.

Materials and Methods

Thirty mongrel dogs of either sex, weighing 7–16 kg, were anesthetized with sodium pentobarbital (30 mg/kg, i.v.). After treatment with sodium heparin (200 units/kg, i.v.), dogs were sacrificed by rapid exsanguination from the right common carotid artery. The internal carotid arteries (outer diameter of 1.0–2.0 mm and 7–20 mm in length) were carefully isolated from the carotid sinus to the carotid foramen. The internal carotid artery gave off no branch within this distance; thus, a single flow stream from the inserted stainless steel cannula passing the intraluminal side was ensured (Fig. 1). A stainless steel cannula was prepared with 2 or 3 small holes at 5 mm distance from the distal blind end (21, 23, 25 or 27 gauge.; outer diameter of 0.40–0.83 mm and 3 cm in length). Each artery was cannulated with a needle which was slightly thinner than the artery in order to obtain an adequate perfusion pressure (100±20 mm Hg). The stainless steel cannula was carefully inserted into each isolated artery to avoid any injury to the internal surface of the vessel. The proximal part of each isolated artery was tied to the stainless steel needle; thus, the stream from the holes of the needle passes only through the intraluminal surface of the isolated artery.
The time interval from the isolation to the perfusion of the artery was usually 1–2 hr. The cannulation procedure was performed in cold Ringer’s solution at a temperature of 4–10°C. As indicated by the arrows (Fig. 2), the stainless steel cannula and the artery were placed in a cup-shaped glass container and perfused with mammalian Ringer’s solution (constituents of which are: 9.0 g NaCl, 0.42 g KCl, 0.24 g CaCl₂, 0.30 g NaHCO₃ per 1000 ml distilled water) by means of a peristaltic pump (Harvard Apparatus, model 505-1210). The solution was bubbled with a mixture of 95% O₂ and 5% CO₂ and maintained at constant temperature of 37°C. The bath was warmed with a circulator pump (Taiyo thermopump Co.) at a constant temperature of 37°C. The flow rate (1.2–7.2 ml/min) was determined at the beginning of the experiment for values inducing a resting perfusion pressure of approx. 100 mm Hg. The perfusion pressure was measured with an electric manometer (Nihon Kohden Co. RP-2), and vasoconstriction was presented as an increase in perfusion pressure. Before commencing the experiments, the preparations were allowed to equilibrate for over 30 min in the bathing
medium. The interval of drug injection was not less than 5 min in order to avoid tachyphylaxis. The volume of drug solution in a single shot was 0.01–0.03 ml that was injected by a microinjector (Terumo Co.), and the injection time was 2–4 sec.

Drugs used were serotonin creatinine sulfate (5-hydroxytryptamine, Sandoz), dl-norepinephrine hydrochloride (Sankyo), methysergide (Sandoz), phentolamine mesylate (Ciba) and potassium chloride.

The data are presented as the mean±S.E.M. in the text and illustrations. An analysis of variance for repeated measures was used to evaluate any differences in the responses among the groups. Student’s unpaired t-test or a t-test for paired data was used, and a P value of 0.05 was considered significant.

Results

1 Constrictor responses of the internal carotid artery to 5-HT and norepinephrine: When 5-HT was given intraluminally to the internal carotid artery of the dog, an immediate increase in perfusion pressure was observed in all the experiments. The threshold dose for inducing a vasoconstriction was approx. 0.02 μg, ranging from 0.001 to 0.1 μg. The maximum increase in perfusion pressure by 5-HT was over 200 mm Hg at the approximate dose range of 0.1–1 μg. The response to 5-HT was repetitively induced when the injection interval was longer than 5 min and when the increase in perfusion pressure was approx. 100 mm Hg. On the other hand, norepinephrine induced a relatively small increase in perfusion pressure even at extremely high doses. In 2 out of 11 preparations, norepinephrine caused no significant vasoconstrictor effect at a dose range of 10–100 μg. The threshold dose for inducing vasoconstriction was approx. 3 μg; and at a large dose of 10 μg, norepinephrine induced a maximum increase in perfusion pressure of about 20 to 40 mm Hg.

Figure 3 shows the results of an experiment with 5-HT and norepinephrine in the same preparation. Summarized data are shown in Fig. 4.

2 Effects of methysergide on 5-HT-induced vasoconstrictor response of the internal carotid artery: When methysergide was administered, a slight vasoconstrictor response was usually observed. Methysergide induced an increase of 3.2±1 mm Hg (mean±S.E.M.) in perfusion pressure at 10 μg (n=11) and 13±6 mm Hg at 100 μg (n=14). A single injection of methysergide at doses of 10 and 100 μg significantly antagonized the 5-HT-induced vasoconstriction. Figure 5 shows the blocking effect of 100 μg of methysergide on 5-HT-induced vasoconstriction. Summarized data are shown in Fig. 6. The blocking action of methysergide at 10 μg continued over a 30 min period.

3 Effects of methysergide on norepinephrine-induced vasoconstrictor response: After treatment with methysergide, the vaso-
constrictor response to norepinephrine was clearly enhanced in all experiments. Figure 7 shows a typical tracing of the effect of 100 μg of methysergide on the norepinephrine-induced vasoconstriction. Summarized data are shown in Fig. 8. On the other hand, norepinephrine-induced vasoconstriction was significantly inhibited by treatment with an alpha-adrenoceptor blocking agent, phentolamine (Fig. 9).

4 Effects of methysergide on potassium chloride-induced vasoconstrictor response: When potassium chloride was administered into the internal carotid artery, an increase in perfusion pressure was usually observed at doses of 1–10 mg. Figure 10 shows that the constrictor response to 3 mg of potassium chloride is potentiated by 100 μg of methysergide. The vasoconstriction was significantly

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Fig. 5. Blocking effect of 100 μg of methysergide on 5-HT-induced vasoconstriction in the isolated internal carotid artery of the dog.

Fig. 6. Effects of methysergide on 5-HT-induced vasoconstriction of the internal carotid artery of the dog. Vertical bars represent the mean ± standard errors.

Fig. 7. Potentiating effects of 100 μg of methysergide on increasing doses of norepinephrine in the internal carotid artery of the dog.

Fig. 8. Effects of methysergide on norepinephrine-induced vasoconstriction in the internal carotid artery of the dog. Vertical bars represent the mean ± standard errors.
Discussion

In the present experiments, we confirmed that the stainless steel cannula inserting method is very useful for observing vascular reactivity by vasoactive substances as previously reported by Hongo and Chiba (1). The results of this study showed that methysergide caused a slight vasoconstriction in the internal carotid artery of the dog. Since it is reported that methysergide has a 5-HT-like property (8-10), the vasoconstrictor action may be mediated via stimulation of 5-HT receptors.

Since the internal carotid artery may contribute to the intracerebral circulation, the changes in this arterial tone may influence the cerebral circulation. In 1978, Chiba et al. (2) reported that 5-HT produced a potent vasoconstriction in the internal carotid artery by use of an isolated and blood-perfused arterial preparation. In this study, we confirmed that 5-HT caused a potent vasoconstrictor response in this artery. 5-HT-induced vasoconstrictor responses were significantly inhibited by treatment with methysergide as reported already (11). In 1980, Lamar and Edvinsson (12) reported that intracranial arteries stimulated by 5-HT were more sensitive to methysergide than extracranial arteries; in intracranial vessels, the methysergide antagonism is non-competitive, while in extracranial vessels, it is competitive. Thus, they reported that the differences of the responses between intracranial and extracranial vessels might suggest a heterogeneity of 5-HT receptors located within the cranial circulation. Therefore, the nature of the internal carotid artery of the dog might be similar to the intracranial vessels.

In this study, methysergide caused an enhancement of norepinephrine-induced vasoconstrictor action. In 1967, Walsh (11) reported that methysergide did not affect the vasoconstrictor actions of norepinephrine and epinephrine in the upper limb vessels of 2 volunteers. In 1972, Saxena (13) reported...
that there was an enhancement in the response to norepinephrine by methysergide in the external carotid artery in vivo. However, it has also been reported that the norepinephrine-induced vasoconstriction was not changed after methysergide in the baboon internal and external carotid arteries in vivo (7). In 1976, Fozard (14) reported that methysergide caused an increase in sensitivity of the rabbit auricular artery to norepinephrine, but not to potassium chloride, suggesting that the sensitization is not due to activation of the inward calcium current, but due to activation of the contractile process. However, in the present study we demonstrated that methysergide potentiated the responses not only to norepinephrine but also to potassium chloride. Although the mechanisms of the potentiating effect of methysergide on the norepinephrine-induced vasoconstriction has not been understood in detail, two possible speculations are as follows: 1) Methysergide may produce an enhancement of the action of norepinephrine due to stimulation of the inward calcium channel. 2) Methysergide may block the uptake of catecholamines into storage sites in the sympathetic nerve endings, like the action of cocaine as has been observed in the cat spleen (15).

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