COMPARATIVE STUDIES OF CEREBRAL VASODILATORS ON RELAXATION ACTIVITIES IN ISOLATED BASILAR, MESENTERIC AND PULMONARY ARTERIES OF RABBITS

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Abstract—Effects of cerebral vasodilators such as bencyclane, cinnarizine, and papaverine were comparatively studied using helically cut basilar and superior mesenteric arteries and radial muscle preparations of pulmonary arteries with the sympathetic nerve isolated from rabbits. The order of relaxation activities on high K+-induced contractures was cinnarizine > bencyclane > papaverine in basilar strips and cinnarizine > papaverine > bencyclane in mesenteric strips. Relaxation responses of basilar strips to cinnarizine and bencyclane were faster and more marked than those seen in mesenteric strips. Responses to papaverine were equipotent in both preparations. The action of cinnarizine alone was irreversible. In mesenteric strips, the order of the sensitivity of contractile responses to cumulatively applied biogenic amines was serotonin > noradrenaline > histamine. Cinnarizine produced an antihistaminergic action, while bencyclane produced an antiserotonergic action. In pulmonary arteries, $6 \times 10^{-6}$ g/ml papaverine inhibited contractile responses to 2, 5, and 25 Hz nerve stimulation in a frequency-independent manner together with inhibition of responses to noradrenaline. Bencyclane at $6 \times 10^{-6}$ and $10^{-5}$ g/ml selectively inhibited in a dose-dependent manner contractile responses only to 25 Hz without inhibition of responses to noradrenaline. These results were discussed in comparison with findings of the cerebral vasodilators obtained using other experimental techniques. Spiral strips of basilar arteries from rabbits in combination with peripheral arteries may be used as a simple, quantitative, and reproducible screening method in a preclinical stage for drug evaluation of cerebral vasodilators.

Bencyclane, 3-[(1-benzylcycloheptyl)oxy]-N,N-dimethylpropylamine fumarate, is a cerebral and peripheral vasodilating agent which produces a relaxation of various kinds of smooth muscles (1–3), slight and transient hypotension (1, 4), inhibition of thrombus formation, and acceleration of fibrinolytic activity of the blood (5, 6). Data which suggest the improvement of cerebral circulation by bencyclane have been accumulated using various approaches and different species (4, 7–10).

Concerning the responsiveness of isolated
arteries, numerous studies have suggested that cerebral and peripheral arteries respond differently to vasoconstricting agents in dogs (11,12), cats (13), and rabbits (14). However, there is a paucity of data regarding the effects of vasodilators on cerebral vessels isolated from rabbits. In the present experiments, we attempted to establish a new simple preclinical screening method for cerebral vasodilators, and the actions of bencyclane were quantitatively analyzed on high K+-induced contractures of helical strips of basilar and superior mesenteric arteries, on contractile responses of mesenteric arteries to some biogenic amines, and on contractile responses of radial muscle preparations of pulmonary arteries to sympathetic nerve stimulation. Findings were compared with those of well-known cerebral vasodilators, cinnarizine and papaverine.

MATERIALS AND METHODS

Spirally cut arterial strips: The strips were prepared according to the method described by Toda (15). Rabbits of either sex, weighing 1.8 to 3.3 kg, were sacrificed by bleeding from the common carotid arteries. The brain and the distal portion of the superior mesenteric arteries (approx. 0.5 mm outside diameter) were isolated. The basilar artery (0.3 mm) was removed from the brain, and arteries were helically cut at an angle of approx. 45° to the longitudinal axis into strips (approx. 25×2 mm in the mesenteric artery and 18×1 mm in the basilar artery). The spiral strips were fixed vertically between hooks in a 20 ml bath containing Ringer-Locke solution. The resting tension was adjusted to 0.5 g in the basilar artery and to 1.5 g in the mesenteric artery. The bathing solution was bubbled with 95% O₂ and 5% CO₂ and was maintained at 37±1°C. The pH of the solution was 7.2 to 7.4. The composition of the solution was as follows (mM): NaCl, 147.2; KCl, 5.4; CaCl₂, 2.2; NaHCO₃, 14.9; and glucose, 5.6. Before the start of the experiments, the preparations were allowed to equilibrate for 2 hr in the medium. During the equilibration period, the solution was replaced every 20 min.

After equilibration of the contractures of arterial strips induced by high K⁺, a vasodilating drug was cumulatively added. Dose-response curves were expressed as relative % values to the maximum relaxation induced by the final application of papaverine. Median effective dose (ED₅₀) was calculated as the concentration which produced 50% of the maximum relaxation induced by each vasodilator before final application of papaverine.

Mesenteric strips were also used to study the interactions between vasodilators and biogenic amines. Dose-response relationships were observed several times by adding the amine cumulatively and then by washing the strips with fresh solution until 2 successive and comparable dose-response curves to the amine were obtained. An equilibration period of 20 min was allowed after washing. Vasodilators were added 20 min before the final challenge with amines. Dose-response relationships were calculated as the relative % values to the maximum contraction induced by each amine immediately before the applications of vasodilators. The pA₂ and pD₂' values were calculated by the method described by Van Rossum (16).

Radial muscle preparations of the pulmonary arteries with sympathetic nerves: These preparations were prepared from rabbits according to the method described by Bevan (17), and they were suspended in Krebs bicarbonate solution of the following composition (mM): NaCl, 118.4; KCl, 4.7; CaCl₂, 2.5; MgCl₂, 1.18; NaHCO₃, 25; KH₂PO₄, 1.2; and glucose, 11.1. The resting
tension was 1.5 g and the equilibration period was 2.5 to 3 hr. The nerve was stimulated through a suction electrode for 15 sec at 4 min intervals using rectangular pulses of 2 msec and 10 V at a frequency of 2, 5 or 25 Hz generated by an electrical stimulator, MSE-3R (Nihon Kohden). Vasodilators were added only after 2 successive and comparable frequency-response relationships had been obtained. Contractile responses to cumulatively applied noradrenaline were also observed as in the mesenteric strips.

Relaxation and contraction responses of arterial preparations were displayed on an ink-writing polygraph, MR 150 (Nihon Kohden).

Drugs used were bencyclane fumarate (Sumitomo), cinnarizine (Eisai), methysergide (Sandoz), isoxsuprine hydrochloride (Daiichi), phenoxybenzamine hydrochloride (Tokyo Kasei), propranolol hydrochloride (ICI), atropine sulfate, papaverine hydrochloride, procaine hydrochloride, diphenhydramine hydrochloride, noradrenaline bitartrate, serotonin creatinine sulfate and histamine dihydrochloride (Sigma). Noradrenaline was dissolved in 0.06 N HCl, 20 mg cinnarizine was dissolved in 20 ml of distilled water containing 50 mg tartaric acid and 9 mg NaOH, and other drugs were dissolved in saline. The maximum volume of cumulatively applied drugs was 1 ml. The concentrations of drugs are usually expressed as g/ml except in the calculation of pA₂ and pD₂' values. The Student’s t-test was used to evaluate data.

RESULTS

Effects of cerebral vasodilators on K⁺-induced contractures of rabbit basilar and superior mesenteric arteries: Contractures were induced promptly after application of 18 to 28 mM K⁺. The plateau was reached by approx. 10 min after the addition in basilar strips and by 20 min in mesenteric strips and

![Rabbit Basilar Artery](image)

Fig. 1. Typical effects of cerebral vasodilators on high K⁺-induced contractures of rabbit basilar strips. Horizontal lines at left ends of traces show tension levels before contractures induced by high K⁺, 18 to 28 mM. Drugs were cumulatively added at each dot. Vertical and horizontal bars show tension and time scales.
persisted for at least 2 hr. Contractile tension was 0.3 to 0.4 g in the former and 1.3 to 1.7 in the latter. In basilar strips (Fig. 1), relaxation responses to bencyclane and papaverine were seen within 1 min, and a plateau was reached 2 to 3 min after the addition of each concentration. The time to the maximum relaxation was 10 to 20 min. On the other hand, responses to cinnarizine developed slowly and 25 to 40 min was required to reach the maximum relaxation. The respective maximum relaxation induced by the 3 vasodilators reached a level below the initial tension. As shown in the lower half of Fig. 2, the order for the intensity of relaxation activities was cinnarizine (0.04) > bencyclane (1.1) > papaverine (2.5). ED50 values (×10^-6) are indicated in the parentheses. Cinnarizine solvent did not modify K⁺-induced contractures.

On the other hand, in strips of the mesenteric artery, the onset of relaxation responses was similar to that in basilar strips, but the time course was slower. Approximately 30 min was required to reach the maximum relaxation in the case of bencyclane and 30 to 50 min for the other vasodilators. The order for intensity of relaxing activities was cinnarizine (0.81) > papaverine (2.7) > bencyclane (4.8) > isoxsuprine, a peripheral vasodilator, (12.5) > phenoxybenzamine and procaine (upper half of Fig. 2). The maximum relaxation induced by papaverine alone reached a level below the initial tension.

Relaxation responses of basilar strips to cinnarizine were significantly more marked than those seen in mesenteric strips (P<0.01, at each dose). Relaxation actions of bencyclane were also more marked in basilar strips than those obtained in mesenteric strips (P<0.05, at each dose except 3×10^-5). The potency ratio in both types of strips was 17.2 for cinnarizine and 4.4 for bencyclane, respectively. On the other hand, relaxation responses to papaverine were equipotent in both preparations; the potency ration was 1.1.

The relaxation action of bencyclane was not affected by 20 min prior treatment with 5×10^-7 g/ml propranolol, atropine and diphenhydramine, respectively, in the mesenteric strips and with 3×10^-7 g/ml of methysergide in basilar strips. These results in combination with those for phenoxybenzamine and procaine suggest that the main action of bencyclane is the direct inhibitory action on vascular smooth muscles and as such is consistent with findings obtained in the other smooth muscle preparations (1–3). With the exception of cinnarizine, the relaxation induced by the other vasodilators used was readily reversed by washing with fresh normal solution. In the case of cin-
narizine, even repetitive washings restored K+-induced contractures only to 0 to 40% of control 3 hr after the 1st washing.

Effects of cerebral vasodilators on contractile responses of rabbit pulmonary arteries to exogenously applied noradrenaline and to sympathetic nerve stimulation: The radial muscle preparations of pulmonary arteries contracted in response to low concentrations of noradrenaline ranging from $4 \times 10^{-9}$ to $2 \times 10^{-8}$ g/ml (Fig. 3). The onset of the action was 8 to 10 sec after the addition of each cumulative dose and the peak tension was obtained within 1 to 2 min. Contractile responses increased in a dose-dependent manner and $4 \times 10^{-5}$ g/ml produced maximum contractions which were obtained 10 to 12 min after the challenge with the lowest concentration of noradrenaline. On the other hand, contractile responses to sympathetic nerve stimulation were dependent on frequencies and were maximum with 25 Hz stimulation. Responses to 40 Hz were the same to those to 25 Hz. However, smaller responses were obtained with increasing frequencies. Bencyclane ($6 \times 10^{-6}$ and $10^{-5}$ g/ml) produced no inhibition of responses to exogenously applied noradrenaline, but selectively inhibited in a dose-dependent manner contractile responses to 25 Hz stimulation 30 min after its addition ($P < 0.05$, $P < 0.01$, compared to control, respectively) (Figs 3 and 4). Stimulation with lower frequencies produced no inhibition until at least 1 hr after the addition. On the other hand, $10^{-6}$ g/ml papaverine produced no inhibition of both responses and a dose of $6 \times 10^{-6}$ g/ml significantly inhibited responses to nerve stimulation in a frequency-independent manner at 2 Hz ($P < 0.05$, compared to control) and at 5 and 25 Hz ($P < 0.01$) concomitantly with moderate inhibition of responses to noradrenaline. Although not shown in Fig. 4, $3 \times 10^{-6}$ g/ml cinnarizine also produced a similar type of inhibition of both responses to that seen with the high dose of papaverine.

Effects of bencyclane and cinnarizine on contractile responses of rabbit superior mesenteric arteries to noradrenaline, serotonin and histamine: Mesenteric strips responded to low concentrations of noradrenaline

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**Fig. 3.** Typical effects of bencyclane on contractile responses of radial muscle preparations of rabbit pulmonary arteries to cumulatively applied noradrenaline (upper trace) and to sympathetic nerve stimulation (lower trace). The numbers 1 to 8 under the upper trace indicate concentrations of noradrenaline (NA) of $10^{-9}$, $4 \times 10^{-9}$, $2 \times 10^{-8}$, $10^{-7}$, $4 \times 10^{-7}$, $2 \times 10^{-6}$, $10^{-5}$, and $4 \times 10^{-5}$, respectively. Preparations were washed with fresh normal Krebs solution after which bencyclane was added at the arrow 20 min before the final challenge of noradrenaline. At the horizontal bars under the lower trace, the sympathetic nerve was stimulated with a frequency of 2, 5, and 25 Hz at 4 min intervals. Bencyclane was added at the arrow after control nerve stimulation. Other details are as in Fig. 1.
ranging from $10^{-9}$ to $4 \times 10^{-9}$ g/ml. The onset of the action was 4 to 5 sec and the time required to reach a plateau was 30 to 80 sec after the addition of each cumulative dose. Contractile responses increased in a similar dose-dependent manner to those in the radial muscle preparations of pulmonary arteries (Fig. 3). However, the maximum contraction in mesenteric strips was observed with $10^{-5}$ g/ml approx. 5 min after the challenge with the lowest dose of noradrenaline. The order of the sensitivity of mesenteric strips to biogenic amines tested was serotonin > noradrenaline > histamine (Fig. 5). The time course of contractile responses to serotonin or histamine was much the same as that to noradrenaline. Doses of histamine over $10^{-4}$ g/ml produced relaxation in this preparation.

**Noradrenaline:** The pretreatment with bencyclane at $3 \times 10^{-6}$ to $3 \times 10^{-5}$ g/ml reduced in a dose-dependent manner the maximum height of the log-dose-response curves for noradrenaline, indicating that the antagonism is noncompetitive at the concentrations studied. The pD$_2$' value was 4.3. On the other hand, cinnarizine ($10^{-7}$ g/ml) produced a slight parallel displacement of the curve to the right, indicating a competitive antagonism (pA$_2$=6.6). Before addition of cinnarizine, equivalent ED50 values were obtained from 2 successive dose-response curves to noradrenaline. Cinnarizine at $10^{-6}$ and $3 \times 10^{-6}$ g/ml dose-dependently reduced the height of the curves (pD$_2$'=5.3).

**Serotonin:** Bencyclane at $10^{-6}$ and $10^{-5}$ g/ml, in a dose-dependent manner, produced an atypical antagonism against serotonin: the slope of each of the curves was flatter at lower doses of serotonin, but there was a tendency for a parallel shift of the curves to the right with higher doses of the agonist. Although the details of the exact mechanism of this antagonism should be clarified, the pA$_2$ value presumptively measured was 7.3. Cinnarizine at $10^{-6}$ g/ml reduced the maxi-
mum amplitude of the curve for serotonin (pD₂' = 5.9).

Histamine: Bencyclane at 5 × 10⁻⁵ g/ml slightly and cinnarizine at 2 × 10⁻⁸ g/ml markedly reduced the maximum height of the curves for histamine. The pD₂' value was 3.8 for bencyclane and 7.8 for cinnarizine, respectively.

The antagonistic actions of bencyclane against these amines were reversed by washings with fresh normal solution. The actions of cinnarizine were irreversible.

DISCUSSION

Rabbit basilar arteries have a smaller diameter and a thinner vessel wall than do superior mesenteric arteries, and there is little adipose tissue surrounding the basilar vessel. The time course for the relaxation action of cerebral vasodilators tested on high K⁺-induced contractures was faster in basilar strips than in mesenteric strips. Relaxation induced by cinnarizine and bencyclane against K⁺-induced contracture was more marked in the basilar strips. These differences may be derived from the sensitivity of both preparations to the drugs and from the structures of both vessels. However, the relaxation responses to papaverine showed no differences related to the preparations. The actions of cinnarizine were persistent and this is consistent with findings of prolonged marked increases in coronary blood flow in dogs (18). The order of activity of bencyclane and papaverine was reverse in both basilar and mesenteric arteries. In this respect, various findings are reported as follows: more marked facilitatory actions of bencyclane than those of papaverine were observed on blood flow in dog carotid arteries assessed by an electromagnetic flow meter (4) and similar dilating activities of bencyclane and papaverine on K⁺-induced contractures of isolated coronary arteries of cattle (2) and on the diameter of rat arteries in the cerebral cortex were observed under the light microscope (9). The present results regarding bencyclane and cinnarizine are consistent with findings of other investigators as follows: bencyclane-induced improvement in changes of metabolic parameters caused by arterial hypoxemia in dogs (10); bencyclane-induced increases in the content and uptake of glucose in rat brain, in tolerance to anoxia in mice and...
in the blood supply of dog brain estimated by changes of cerebrospinal fluid pressure (8); bencyclane-induced increases in the blood flow of the total brain and of the ischemic area in humans measured by the $^{133}$Xe clearance method (7); and cinnarizine-induced improvement of cerebral circulation estimated by the $^{131}$I-hippuran method in humans (19). Dogs are mainly used when attempting to prepare isolated spiral strips of the cerebral arteries (11, 12, 15). However, preparing helical strips of basilar arteries was easy even in rabbits weighing 1.8 to 2.0 kg.

Relatively high concentrations of bencyclane inhibited in a frequency-dependent manner contractile responses of pulmonary arteries to sympathetic nerve stimulation without inhibiting responses to exogenously applied noradrenaline. This finding shows the selectivity of the presynaptic site of bencyclane action. On the other hand, papaverine and cinnarizine produced no such type of selectivity. The mechanism of the frequency-dependent inhibition may be attributed to the local anesthetic property of bencyclane (1) because local anesthetics generally produce this type of inhibition in various neuronal systems (20–23).

The major interaction between bencyclane and the biogenic amines tested was an antiserotonergic action in superior mesenteric arteries, and this result is consistent with the findings in stomach fundus contraction, anaphylactoid reactions and permeability of skin of rats and dyspnoea of guinea pigs (24) and in rat blood pressure (25). On the other hand, cinnarizine produced a marked non-competitive antihistaminergic action; and this is consistent with the results obtained in ileum contraction, anaphylactoid reactions, permeability of skin, acute toxicity in guinea pigs (26), and with the antiallergic action in man (27). Non-competitive antinoradrenergic actions of bencyclane were not so marked. and this finding is consistent with data on guinea-pig Langendorff preparations (2).

It can be concluded that the spiral strips of basilar arteries of rabbits in combination with peripheral arteries may be used as a simple, quantitative, and reproducible screening method in a preclinical stage for drug evaluation of cerebral vasodilators.

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