EFFECTS OF BROVINCAMINE ON THE AUTONOMIC NERVOUS FUNCTION IN THE DOG AND RAT

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Effects of brovincamine (BV) on peripheral nerves were studied in dogs and rats. 1) In pentobarbital-anesthetized dogs, BV (6.4 mg/kg) administered i.v. did not affect the changes of blood pressure and heart rate induced by noradrenaline and adrenaline, but slightly inhibited the hypotensive effect of acetylcholine. BV had no effect on the hypertension elicited by carotid sinus reflex, but diminished the bradycardia by vagus nerve stimulation. BV did not influence the tachycardia elicited by stimulation of the postganglionic nerve to stellate ganglion, but slightly inhibited that by the preganglionic stimulation. 2) In spinal dogs, BV (6.4 mg/kg) given i.v. slightly inhibited the increases of blood pressure and heart rate induced by i.v. dimethylphenylpiperazinium (DMPP). When i.v. administered in divided doses of 2 and 4 mg/kg, BV induced a slight but stepwise inhibition of the tachycardia elicited by direct administration of DMPP, betahanechol (BCH) and angiotension II (AT II) to the cardiac sympathetic ganglia via the subclavian artery. 3) In rat isolated diaphragm nerve preparations, BV at $10^{-6}$ and $10^{-4}$ g/ml dose-dependently reduced the twitch response to nerve stimulation. 4) In conclusion, BV does not affect the sympathetic activities but inhibits the cholinergic function in the autonomic nervous system.

Keywords—brovincamine; peripheral nervous system; cholinergic inhibition; sympathetic ganglionic inhibition; dog; rat

INTRODUCTION

Vincamine, an alkaloid extracted from *Vincam minor* L, has been used as a remedy for cerebrovascular disorders and for sequellae of head injury,1,2) since it brings about a decrease of vascular resistance and increase of the cerebral blood flow.3,4) Brovincamine (BV) is a new compound to which bromine is introduced at the position 11 of vincamine, and has an advantage over vincamine in the intestinal absorption. From our previous experiments, BV was proven to have a vasodilatory effect on the cerebral and coronary vessels, in which the direct action on blood vessels, blockade of the depolarization-dependent Ca²⁺ channel, seems to be involved.5) Besides, the clinical double blind trials show that BV is effectively applicable to the treatment of cerebrovascular disorders.

In this study, we further investigated effects of BV on the autonomic nervous system.

MATERIALS AND METHODS

General—Mongrel dogs of either sex weighing between 6 and 12 kg were anesthetized with 30 mg/kg of pentobarbital sodium administered i.v. A tracheal cannula was inserted at the midcervical level. Blood pressure was measured with a pressure transducer (MPU-0.5-290-11, Nihon Kohden) connected to a cannula placed in the right femoral artery. Heart rate was obtained with a tachograph (AT-600G, Nihon Kohden) which was triggered by the femoral arterial pulse wave. The output signals from the pressure transducer and the cardiograph were both recorded on an ink-writing polygraph (RM-6000, Nihon Kohden).

Vagus Nerve Stimulation and Carotid Sinus Reflex — The right vagus nerve was cut at the cervical region, and the severed distal end of the nerve was placed on a bipolar platinum electrode and stimulated with rectangular pulses (1, 2 and 4
Hz frequencies, 0.1 ms duration, 5 V intensity and 5 s period) produced by an electric stimulator (MSE-3R, Nihon Kohden). The carotid sinus reflex was elicited by bilateral carotid occlusion for 30 s using cramps.

Pre- and Postganglionic Stimulation of Stellate Ganglia — The surgical procedures were performed principally according to the methods described by Flacke and Gillis. The animals were respirated with air by means of a Harvard respirator (Model 607, Harvard) (24 strokes/min, 300 ml tidal volume). To eliminate reflex changes in autonomic activity, both vagus nerves were cut in the midcervical region and both carotid arteries were ligated. In addition the spinal cord was severed through the atlantooccipital foramen with a scalpell, and the foramen was quickly sealed with a cork. After the chest was opened by severing the upper three ribs, the stellate ganglion area on the right side was exposed and the ansa subclavia (the preganglionic nerve to the stellate ganglion) was dissected and cut. A bipolar platinum electrode was placed on either the distal end of the ansa subclavia or the stellate cardiac nerve (the postganglionic nerve to the stellate ganglion), which was stimulated with rectangular pulses (1, 2 and 4 Hz frequencies, 1 ms duration, 40 V intensity and 10 s period) produced by an electric stimulator (MSE-3R, Nihon Kohden).

Since blood pressure was decreased after section of the spinal cord, the pressure was maintained at about 60–70 mmHg during the experiment by an infusion of dextran (6% in saline contained 5% glucose) into the femoral vein.

Drug Administration via Subclavian Artery to the Cardiac Sympathetic Ganglia — The surgical procedures in this experiment were performed principally according to the methods described by Fleisch et al. Similarly to the method mentioned above, both vagus nerves in the midcervical region and spinal cord were severed, and both carotid arteries were ligated under artificial ven-

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**FIG. 1. Influence of BV on the Changes in Blood Pressure Induced by i.v. Noradrenaline, Adrenaline and Acetylcholine in Dogs**

Before BV (□), 30 min (□) and 120 min (□) after BV (6.4 mg/kg, i.v.). Each column represents the mean of 6 animals. Vertical bars show standard error of the mean. NA: noradrenaline, A: adrenaline, ACh: acetylcholine. a) Denotes significant difference between values before and after BV in the same animals.
tilation. After the right chest was opened by severing the upper three ribs, the right internal mammary, vertebral and superficial cervical arteries were ligated. The right brachial artery was then cannulated with a polyethylene catheter connected to a stopcock. The catheter was advanced proximally until the tip was approximately at the junction of the right internal mammary and subclavian arteries, and was tightly secured in place. After completion of the surgical procedure, the animals were treated with 300 units/kg of heparin. The ganglionic stimulants, such as DMPP, BCH and AT II, were rapidly injected i.a. in a volume of 0.1 ml through a catheter inserted into the subclavian artery. BV was injected into the right femoral vein through a catheter. For dose-response studies with ganglionic stimulants, about 20 min were allowed after divided doses of i.v. BV (2 and 4 mg/kg) for another dose-response run.

**Neuromuscular Transmission in Isolated Diaphragm** — Male Wistar rats weighing between 250 and 300 g were killed by a blow to the head and bleeding. The diaphragm with phrenic nerve was removed, and neuromuscular preparation was made according to the method of Bülburing. The preparation suspended in Krebs-Ringer solution at 37°C in a 10 ml bath which was bubbled continuously with 95% O₂ and 5% CO₂. The phrenic nerve was continuously stimulated electrically with 6 V square wave pulses, 1 ms in duration, delivered at a frequency of 0.2 Hz from an electric stimulator (MSE-3R, Nihon Kohden) connected to silver electrode. Contraction of the diaphragm was measured by a strain guage (VC-2, Keisoku) and recorded on a chart recorder (RM-6100, Nihon Kohden). Drug was cumulatively applied into the chamber at intervals of 5 min after the twitch response elicited by nerve stimulation reached to a constant.

**Drugs** — Brovincamine hydrogen fumarate (BV, Sandoz), dl-noradrenaline hydrochloride (Sankyo), dl-adrenaline hydrochloride (Daichi Pharmaceutical), acetylcholine chloride (Daichi Pharmaceutical), 1,1-dimethyl-4-phenylpiperazinum iodide (DMPP, Aldrich), betanachol chloride (BCH, Eizai), vaL-angiotensin II (AT II, Protein Research Foundation), heparin sodium (Novo) and dextran (clinical grade Dextran D, Ohtsuka Pharmaceutical) were used in this study.

BV was dissolved in 1% tartaric acid in a concentration of 1% solution. This solution was about 2.7 in pH. All doses were expressed in terms of the base.

**Statistical Analysis** — Each value represents the mean ± S.E. Comparisons of values before and after BV in the same animals were made by paired t-test. Values were considered to be significantly different at the level of p < 0.05.

**RESULTS**

**Effects on Cardiovascular Action of Noradrenaline, Adrenaline and Acetycholine**

Results are shown in Figs. 1 and 2. In 6 dogs (mean blood pressure, 126 ± 5.5 mmHg and heart rate, 152 ± 11.9 beats/min), noradrenaline and adrenaline given i.v. at doses of 1 and 2 µg/kg

**FIG. 2. Influence of BV on the Changes in Heart Rate Induced by i.v. Noradrenaline, Adrenaline in Dogs**

*Before BV (□), 30 min (□□) and 120 min (□□□) after BV (6.4 mg/kg, i.v.). Each column represents the mean of 6 animals. See legend to Fig. 1 for additional explanations.*
induced a rise of blood pressure and biphasic changes of heart rate, *i.e.* an increase followed by a slight decrease. These effects of the amines were unaffected 30 and 120 min after administration of BV. Acetylcholine administered *i.v.* at the same doses induced a transient hypotension without an obvious change in heart rate, which was slightly inhibited by pretreatment with BV.

**Effects on Carotid Sinus Reflex and Vagus Nerve Stimulation**

In 6 dogs (mean blood pressure, 124±6.2 mmHg and heart rate, 160±7.7 beats/min), blood pressure was lowered to 116±5.6 and 117±8.2 mmHg, and heart rate decreased to 143±7.9 and 149±6.5 beats/min, respectively, 30 and 120 min after *i.v.* administration of BV at a dose of 6.4 mg/kg. The bilateral carotid occlusion for 30 s induced a blood pressure increase and this increase was not affected 30 and 120 min after BV, as shown in the left panel of Fig. 3.

In 6 dogs (blood pressure, 143±9.2 mmHg and heart rate, 159±10.5 beats/min), bradycardia was caused by stimulation of the right vagal nerve at frequencies of 1, 2 and 4 Hz, as seen in the right panel of Fig. 3. When heart rate decreased to 147±7.6 and 151±8.7 beats/min, respectively, 30 and 120 min after *i.v.* administration of BV, the frequency-dependent bradycardia by the vagus stimulation was significantly inhibited.

In these two groups of the experiments on carotid sinus reflex and vagus nerve stimulation, the decrements of blood pressure and heart rate

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**FIG. 3.** Influence of BV on the Blood Pressure Rise Elicited by Carotid Occlusion (Left Panel) and the Heart Rate Decrease by Vagus Stimulation (Right One) in Dogs

Before BV (□), 30 min (□□) and 120 min (□□□) after BV (6.4 mg/kg, *i.v.*). Each column represents the mean of 6 animals. See legend to Fig. 1 for additional explanations.
30 or 120 min after administration of BV were insignificant from values before the injection of BV, except for the bradycardia 30 min after the drug.

**Effects on Pre- and Postganglionic Stimulation**

In 6 spinal dogs (mean heart rate, 130 ± 7.0 beats/min), stimuli at 1, 2 and 4 Hz applied to the preganglionic sympathetic nerve or to the postganglionic sympathetic nerve elicited a frequency-related increase in heart rate. Heart rate decreased to 102 ± 10.0 and 107 ± 10.1 beats min at 30 and 120 min after i.v. administration of BV, respectively. As shown in Fig. 4, the rate increase induced by preganglionic stimulation was inhibited 30 min after dosing of BV, but this inhibition recovered to the control level 120 min after the dosing. On the other hand, the rate increase by postganglionic stimulation was not affected after BV.

**Effects on Cardiovascular Responses to DMPP**

In 5 spinal dogs (mean blood pressure, 72 ± 9.6 mmHg and heart rate, 117 ± 9.0 beats/min), DMPP injected i.v. at doses of 5 and 10 µg/kg caused immediate increases in blood pressure and heart rate. As shown in Fig. 5, these effects of DMPP were inhibited 30 min after BV.

**Effects on Ganglionic Stimulant Actions of DMPP, BCH and AT II**

In 7 spinal dogs (mean heart rate, 141 ± 8.2 beats/min), DMPP given at doses of 2.5 – 40 µg to the cardiac sympathetic ganglia through the subclavian artery exerted a dose-dependent posi-

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**Fig. 4. Influence of BV on the Positive Chronotropism Elicited by Pre- and Postganglionic Stimulation of the Right Stellate Ganglion in Spinal Dogs**

Before BV (□), 30 min (□) and 120 min (□) after BV (6.4 mg/kg, i.v.). Each column represents the mean of 7 animals. See legend to Fig. 1 for additional explanations.
tive chronotropic action. Heart rate decreased to 102±6.8 and 83±8.5 beats/min, respectively, after BV administered i.v. in divided doses of 2 and 4 mg/kg. As shown in Fig. 6, BV slightly but dose-dependently inhibited the dose-response curve for DMPP of the positive chronotropic effects.

In 6 spinal dogs (mean heart rate, 137±8.6 beats/min), BCH given i.a. at doses of 2.5—40 μg exhibited a similar dose-dependent positive chronotropic effect. As shown in Fig. 7, BV injected i.v. in divided doses of 2 and 4 mg/kg, which decreased heart rate to 105±9.5 and 85±10.2 beats/min, respectively, slightly inhibited the dose-response curve for BCH without significance.

In another 6 spinal dogs (mean heart rate, 139±10.6 beats/min), as shown in Fig. 8, BV injected i.v. in the same divided doses, which decreased heart rate to 101±6.7 and 86±10.8 beats/min, respectively, significantly reduced the positive chronotropic responses to AT II.

**Effect on Neuromuscular Transmission in Isolated Diaphragms**

Results are shown in Fig. 9. Electrical stimulation of the phrenic nerve at 0.2 Hz induced a twitch response in the isolated rat diaphragm. Solvent at the volume corresponding to BV of 10⁻⁴ g/ml did not affect the twitch response in 2 preparations, while BV (10⁻⁵ and 10⁻⁴ g/ml) pro-

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**FIG. 5. Influence of BV on the Increase in Blood Pressure (Left Panel) and Heart Rate (Right One) Induced by i.v. DMPP in Spinal Dogs**

*Before BV (□), 30 min (☑) and 60 min (☑) after BV (6.4 mg/kg, i.v.). Each column represents the mean of 5 animals. See legend to Fig. 1 for additional explanations. DMPP; dimethylphenylpiperazinium.*
duced a concentration-dependent reduction of the twitch responses, though it did not affect the twitch at $10^{-6}$ g/ml, in each 4 preparations.

DISCUSSION

In our experiments, BV administered i.v. elicited dose-dependent increase of both carotid and vertebral blood flow in dogs and this increase was maximal at a dose of 6.4 mg/kg (unpublished observation). Accordingly, the present study on the autonomic nerve functions was carried out using the same dose of BV, except for study on the sympathetic ganglionic function in which divided dose of 2 and 4 mg/kg was successively i.v. administered.

BV at a dose of 6.4 mg/kg given i.v. caused a transient hypotension and a bradycardia with longer duration. The fall in blood pressure was accounted for the direct vasodilation which seemed to involve an inhibition of depolarization-dependent Ca$^{2+}$ slow channel.5) As for bradycardia, BV at high concentrations ($10^{-5}$ g/ml) exerted a negative chronotropic action in isolated guinea pig atria, suggesting direct action on the heart at large doses (unpublished observation). Vincamine broadened the action potential of the guinea pig sinus node, atrium and papillary muscle, on account of prolonging the repolarization phase and slowed down the spontaneous depolarization of the sinus node.5) While duration of slow action potentials of partially depolarized guinea pig papillary muscle was reduced by exposure to BV,5) Thus, the real mechanisms

**FIG. 6. Influence of BV Administered in Divided Doses on Positive Chronotropic Dose-Response Curve for DMPP in Spinal Dogs**

Before BV (-- ○ --), after BV (2 mg/kg, i.v.) (--- ● ---) and after BV (4 mg/kg, i.v.) (-- - ▲ --). Each point represents the mean of 7 animals. See legend to Fig. 1 for additional explanations.

**FIG. 7. Influence of BV Given in Divided Doses on Positive Chronotropic Dose-Response Curve for BCH in Spinal Dogs**

Before BV (-- ○ --), after BV (2 mg/kg, i.v.) (--- ● ---) and after BV (4 mg/kg, i.v.) (-- - ▲ --). Each point represents the mean of 6 animals. BCH; betanechol. See legend to Fig. 1 for additional explanations.
involved in the BV-induced bradycardia seem to remain to be determined.

On the sympathetic nervous system, BV failed to alter the pressor and positive chronotropic effects of noradrenaline and adrenaline, the pressor response to carotid sinus reflex and the tachycardia elicited by stimulation of postganglionic nerve to the stellate ganglion. It is, therefore, indicated that BV affects neither the presynaptic nor postsynaptic sites in the sympathetic nerve terminals.

On the contrary, BV administered i.v. inhibited the negative chronotropic response to vagus nerve stimulation and the positive chronotropic response to preganglionic stimulation to the stellate ganglion, without affecting that to postganglionic one. In addition, BV reduced not only the cholinergic transmission in the autonomic nervous system but also that in the motor nerve, i.e., the twitch response to the phrenic nerve stimulation of the isolated rat diaphragm. Thus, BV seems to slightly inhibit the cholinergic nerve function.

Besides, intravenous injection of BV slightly inhibited depressor effect of acetylcholine as well as pressor and positive chronotropic effects of DMPP given i.v. BV slightly inhibited the ganglionic stimulant effects of DMPP and BCh administered into the cardiac sympathetic ganglia through the subclavian artery in spinal dogs. These observations suggest that BV also appears to exert its depressant effect on cholinergic function by acting on the postsynaptic sites of parasympathetic nerve ending and ganglia. However, in the experiment on the sympathetic cardiac ganglia, BV inhibited the maximally obtainable response to DMPP. It has been reported that nicotinic antagonists shifted the dose-response curves to nicotinic agonists step-wise to the right without inhibition of maximal response to the agonists on the cardiac sympathetic ganglia of spinal dogs. Therefore, the depressant effect of BV on the ganglionic stimulant response to DMPP is not likely to be due to specific blockade of nicotinic receptors at the postsynaptic sites. This proposal is also supported by findings with AT II. BV dose-dependently inhibited the ganglionic stimulant effect of AT II as well. This ganglionic stimulant effect of AT II

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was unaffected by successive administration of hexamethonium and atropine as well as hemicholinium-3 but was antagonized by saralasin, implying that the stimulant effect is not due to either stimulation of cholinergic receptor at the postganglionic sites or acetylcholine release from the preganglionic nerve terminals, but to the AT receptor at postganglionic sites.\(^{11}\)

For the release of acetylcholine elicited by the nerve stimulation, the presence of calcium ions in the extracellular fluid is essential in the isolated guinea-pig ileum\(^{12}\) and the mouse diaphragm.\(^{13}\) Cholinergic agonists are well documented to produce depolarization and to facilitate calcium ion influx at the receptor sites. Our previous findings show that BV blocks the depolarization-dependent Ca\(^{2+}\) channel.\(^{5}\) Consequently, it is probable that the inhibition of cholinergic function induced by BV is attributable to the reduction of Ca\(^{2+}\) influx.

The present results suggest BV does not affect the sympathetic function but slightly inhibits the cholinergic one of parasympathetic and motor nerves. In fact, BV inhibits the ganglionic activities, but this effect of BV is slight. Accordingly, these effects of BV on the autonomic nervous system seem not to be plausible explanation for its vasodilatory effect. The direct effect on the blood vessels\(^{9}\) appears to be contributed to the vasodilation produced by BV.

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REFERENCES