**IN VIVO DIRECT EFFECTS OF CHOLINERGIC AGENTS ON THE INFERIOR MESENTERIC AND CARDIAC GANGLIA WITH RELATION TO THEIR RECEPTORS IN THE DOG**

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**Abstract**—The relative contribution of nicotinic and muscarinic receptors to the cholinergic transmission of the inferior mesenteric ganglion was studied in spinal dogs by recording changes in perfusion pressure of the inferior mesenteric artery as an indicator of ganglionic function. Preganglionic stimulation elicited a frequency (2.5–320 Hz)-dependent rise in the perfusion pressure, which was inhibited by i.v. hexamethonium (C₆) (10 mg/kg) or atropine (0.1 mg/kg) administered after C₆. Acetylcholine (ACh) (0.1–1000 μg) administered into the inferior mesenteric artery to reach the mesenteric ganglion induced a dose-dependent rise in perfusion pressure and this dose-response curve was shifted to the right by C₆ or atropine. Bethanechol (1–1000 μg) i.a. produced a dose-dependent rise in the pressure, which was abolished after i.v. atropine. Tetramethylammonium (1–300 μg) i.a. elicited an increase in the pressure though the effects were decreased at larger doses, and these effects were strongly inhibited by i.v. C₆. ACh (5–1000 μg) administered into the right subclavian artery to reach the cardiac sympathetic ganglia caused a dose-dependent positive chronotropic effect, which was inhibited by i.a. C₆ or atropine. The results suggest that the inferior mesenteric ganglion seems to differ from the cardiac ganglia in relative contribution of nicotinic and muscarinic receptors to the cholinergic transmission.

The existence in the autonomic ganglia of two cholinergic receptors, nicotinic and muscarinic type, has been demonstrated with different techniques and different ganglia and species, and was reviewed by Volle (1), Trendelenburg (2) and Haefely (3). From investigating functional alterations in the end-organ of the sympathetic ganglion which innervates the cardiovascular system, as an indicator of ganglionic function, Flacke and Gillis (4) and their group (5, 6) have contributed quantitative studies on the nicotinic and muscarinic pathways in the cardiac sympathetic ganglia of the dog. However, there have been few reports which provide information on cholinergic transmission in the sympathetic ganglia which control the vascular tone. The inferior mesenteric ganglion is thought to be a suitable preparation for investigating the ganglionic transmission which innervates peripheral vascular bed.

The aim of the present study was to investigate cholinergic transmission in the inferior mesenteric ganglion of the dog by utilizing changes in perfusion pressure of the inferior mesenteric artery as an indicator of ganglionic function.
MATERIALS AND METHODS

Mongrel dogs of either sex weighing between 8 and 19 kg were anesthetized with 35 mg/kg of pentobarbital sodium administered i.p. The trachea was cannulated and the animals were respired with air by means of a ventilator (KN-50, Natsume) (24 strokes/min, 300 ml tidal volume). Both vagi were cut in the cervical region and the right carotid artery was ligated. In order to eliminate reflex changes in autonomic activity, the spinal cord in all animals was transected with a scalpel through the atlanto-occipital foramen which was quickly sealed with a cork.

The surgical procedures for experiments on the inferior mesenteric ganglion were as demonstrated in Fig. 1. After the left flank was opened, the inferior mesenteric artery was perfused by means of an appropriate tubing placed between the proximal portion of the left carotid artery and the distal portion of the inferior mesenteric artery, using an interposed sigmamotor pump. The flow was adjusted and then pump speed was kept constant during the experiment so that the perfusion pressure approximated the normal blood pressure in the inferior mesenteric artery. The inferior mesenteric artery was then cannulated retrogradely close to the inferior mesenteric ganglion with a polyethylene catheter connected to a stopcock, and the catheter was tightly secured in place.

The surgical procedures for experiments on the cardiac ganglia were performed principally accordingly to the methods described by Fleisch et al. (5). After the chest was opened by severing the ribs between the first and third from the sternum, the right internal mammary and vertebral 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 artery, and was tightly secured in place.

The incisions in the left flank or right chest were covered with a gauze moistened with saline in order to prevent the drying around the ganglia. The temperature in the area of the ganglia was maintained by a heat lamp, between 37 and 39°C. After completion of the surgical procedure, the animals were given 300 units/kg of heparin i.v..

Fig. 1. Diagrammatic representation of the technique used for application of agents into arterial supply to the inferior mesenteric ganglion and for recording vasoconstrictor tone of the inferior mesenteric artery.
Blood pressure and perfusion pressure were measured with a pressure transducer (MPU-0.5-290-0-11, Nihon Kohden) connected to cannula placed in the right femoral or left inferior mesenteric artery. Heart rate was continuously recorded by a cardiotachograph (MT-5, Nihon Kohden). The output signals from the pressure transducer and the cardiotachograph were both recorded on an ink-writing polygraph (RBL-45, Nihon Kohden).

For preganglionic stimulation of the inferior mesenteric ganglion, the left preganglionic nerve to the ganglion was stimulated with supramaximal voltage ranging from 15 to 20 V, a pulse duration of 1 msec and varying frequencies for period of 15 sec using bipolar platinum electrodes and an electric stimulator (MSE-3R, Nihon Kohden).

Since blood pressure was decreased after severing the spinal cord, blood pressure was maintained at about 100 mmHg during the experiment by a constant infusion of dextran (6% in saline containing 5% glucose) into the femoral vein.

In order to avoid outflow of the injected drug solution into the aorta, the agents were slowly injected over 20-30 sec through a catheter inserted previously into the inferior mesenteric artery, while the agents were rapidly injected through a catheter inserted into the subclavian artery as described in the previous report (5). A volume of i.a. injected drugs was from 0.1 to 0.2 ml. Ganglionic blocking agents were given i.v. into the right femoral vein, through a catheter. As control studies, saline (0.1 ml) was given i.a. and the perfusion pressure and heart rate were not affected by these injections.

Drugs employed were; acetylcholine chloride (ACh) (Ovisot, Daiichi), dimethylphenylpiperazinium iodide (DMPP) (K & K), tetramethylammonium bromide (TMA) (Tokyo Chemical), nicotine (Ishizu), bethanechol chloride (Eizai), hexamethonium bromide (C6 (Nakarai), atropine sulfate (Iwaki), pentobarbital sodium (Nembutal, Abbott), heparin (Novo) and dextran (Dextran D, Otsuka).

All doses of drugs were expressed in terms of their bases. A period of approximately 1 to 3 min was allowed to elapse between two frequencies of stimulation or administration of drugs, as perfusion pressure or heart rate returned to the control level during this time. After administration of an antagonist, 5 min were allowed to elapse before another dose-response run was begun. Completion of a run required about 20 min, so that the time interval between dose-response runs was about 25 min.

The statistical significance of the results was calculated by Student’s t-test (p<0.05).

RESULTS

1) Effect of ACh and influence of ganglionic blocking agents in the inferior mesenteric ganglion: Following injection of nigrosine ink dissolved in saline into the inferior mesenteric artery, post mortem macroscopic examination revealed a marked staining of the inferior mesenteric ganglion. Left inferior mesenteric gangliectomy or surgical interruption of the postganglionic nerves of the inferior mesenteric ganglion completely abolished the vasoconstriction in the inferior mesenteric vessel elicited by i.a. administered ACh or preganglionic stimulation, whereas transection of the preganglionic fibers had no effect. Accordingly, in the preparation used in this study, the agents administered into the inferior
mesenteric artery reached the inferior mesenteric ganglion at effective concentrations.

In twenty-two spinal dogs in which perfusion pressure was $89 \pm 5.2$ mmHg, injections of ACh (0.1–250 μg) towards the inferior mesenteric artery elicited a dose-dependent rise in perfusion pressure with a latency of about 3 sec; the peak effect was reached within 10 to 15 sec after the administration and the response lasted for 30 to 40 sec, depending upon the dose. Systemic blood pressure fell with a latency of about 15 sec, the fall being attributed to the peripheral effect of re-circulated ACh. A typical experiment is shown in Fig. 2. The dose-response curve of the ganglionic stimulating effect for ACh is shown in Figs. 3 and 4. The maximal rise in perfusion pressure was obtained with 100 μg of ACh.

In seven experiments, C₆ (10 mg/kg), i.v., lowered the mean perfusion pressure from the control level of $89 \pm 5.2$ mmHg to $66 \pm 2.0$ mmHg, and shifted the dose-response curve for ACh to the right (Fig. 3). Atropine (0.1 and 1 mg/kg) given i.v. after C₆ produced a further shift to the right of the curve to ACh.

On the other hand, as shown in Fig. 4, the i.v. administration of atropine (0.1 mg/kg) before C₆ likewise elicited a shift of the dose-response curve for ACh to the right. In the presence of atropine (0.1 mg/kg), additional doses of C₆ (10 mg/kg) induced a stepwise shift of the curve to the right, but the curve showed a marked increase in the maximal response to ACh after the first administration of C₆.

2) **Effect of preganglionic stimulation and influence of ganglionic blocking agents:**

Preganglionic stimulation of inferior mesenteric nerve at increasing frequencies elicited an

![Fig. 2. Responses of perfusion pressure in the inferior mesenteric artery and systemic arterial blood pressure to i.a. administered acetylcholine, and the effect of hexamethonium and atropine on the responses. The upper panel shows control responses to acetylcholine, the middle panel the responses after hexamethonium and the lower panel responses after additional administration of atropine. BP: systemic arterial blood pressure, PP: perfusion pressure, T: time base marking every second.](image-url)
FIG. 3. Dose-response curve for i.a. acetylcholine in the increase of the inferior mesenteric perfusion pressure, and the influence of hexamethonium and hexamethonium plus atropine. Vertical bars indicate standard errors, and N number of experiments. *, Significant difference from control response to acetylcholine (p < 0.05).

FIG. 4. Dose-response curve of i.a. acetylcholine in the increase of the inferior mesenteric perfusion pressure and the influence of atropine and atropine plus hexamethonium. Further explanations as in Fig. 3.
immediate frequency-dependent rise in perfusion pressure in the inferior mesenteric artery which lasted from 50 to 60 sec depending upon the frequency. There were no changes in the systemic arterial blood pressure, as shown in Fig. 5. The average frequency-response curve for preganglionic stimulation is shown in Fig. 6.

C₆ (10 mg/kg) given i.v. to four spinal dogs lowered the mean perfusion pressure from 79±5.5 mmHg to 70±2.9 mmHg, and strongly inhibited the response to preganglionic stimulation (left panel of Fig. 6). Atropine (0.1 mg/kg), given i.v. after treatment with C₆, produced a further inhibition of the response to preganglionic stimulation, and there was no significant difference from the effects seen with C₆ treatment. On the other hand, as demonstrated in the right panel of Fig. 6, the i.v. administration of atropine (0.1 mg/kg) did not inhibit preganglionic stimulation in another group of experiments, but the subsequent i.v. administration of C₆ (10 mg/kg) eliminated responses to the stimulation.

3) Effects of nicotine, TMA and DMPP and influence of C₆: Nicotine, TMA and DMPP injected into the inferior mesenteric artery elicited a rise in perfusion pressure with a latency of about 5 sec, the rise being maximal 20 sec later and lasting about 1 min in twenty spinal dogs in which the initial perfusion pressure was 103±0.6 mmHg. However, after each injection of successively increasing doses of each agent, the pressor response to high doses decreased. These results are shown in Fig. 7. Thus, the dose-response curve for TMA at doses ranging from 1 to 300 μg was found to have an ascending (1-10 μg) and a

![Fig. 5](image-url)  
**Fig. 5.** Responses of perfusion pressure in the inferior mesenteric artery and systemic arterial blood pressure to preganglionic stimulation at different frequencies, and effect of hexamethonium and atropine on the responses. The upper panel shows control responses, the middle panel responses after hexamethonium and the lower panel responses after additional administration of atropine. Further explanations as in Fig. 2.
Fig. 6. Frequency-response curves for preganglionic stimulation of the inferior mesenteric perfusion pressure and the influence of hexamethonium and atropine. The left panel shows the influence of hexamethonium and hexamethonium plus atropine, the right panel the influence of atropine and atropine plus hexamethonium. *, Significant difference from control response to nerve stimulation; +, Significant difference from the response after atropine alone (p<0.05). Further explanations as in Fig. 3.

Fig. 7. Effects of tetramethylammonium (TMA), nicotine and dimethylphenylpiperazinium (DMPP) injected into the arterial supply of the inferior mesenteric ganglion. The left panel shows effect of TMA and its modification by hexamethonium and right panel effect of nicotine and DMPP. *, Significant difference from control response to TMA (p<0.05). Further explanations as in Fig. 3.
descending limb, the curve reaching the maximum with a dose of 10 \( \mu g \). DMPP, 1-300 \( \mu g \), and nicotine, 10-300 \( \mu g \), produced a gradual decrease in the pressor effect, according to the increase in doses administered.

The TMA-induced rise in perfusion pressure was considerably inhibited after i.v. administration of C6 (10 mg/kg) in three spinal dogs in which the perfusion pressure fell from the control level of 93 ± 10 mmHg to 60 mmHg 5 min after administration of the antagonist.

4) Effect of bethanechol and influence of atropine: The results are summarized in Fig. 8. Bethanechol (1-100 \( \mu g \)), administered i.a. to eight dogs in which the perfusion pressure was 112 ± 10.8 mmHg, caused a dose-dependent rise in perfusion pressure with a latency of about 10 sec, the peak effect being reached within 20 to 30 sec. The response lasted for 20 to 60 sec, depending upon the dose. Systemic blood pressure was lowered after a latency of about 20 sec, presumably because of the peripheral effect of bethanechol. The vasoconstrictive effect of bethanechol was strongly inhibited by i.v. administration of atropine (0.1 mg/kg) in four experiments.

5) Effect of ACh and influence of ganglionic blocking agents in the cardiac sympathetic ganglia: By the method of i.v. injection of the antagonist with the dog cardiac sympathetic ganglia as done in the present experiments on the inferior mesenteric ganglion, the investigations on effects of cholinergic agents and nicotinic and muscarinic receptors involved in the cholinergic stimulation were already reported by Fleisch et al. (5). In the present study, therefore, the antagonists were administered into the subclavian artery to determine the direct effect of ACh on the cardiac ganglia.

As shown in Fig. 9, the injection of ACh (5-1000 \( \mu g \)) into the subclavian artery in thirteen spinal dogs in which heart rate was 150 ± 8.5 beats/min produced an immediate increase in heart rate with a latency of about 2 sec; the peak effect was reached within another

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**FIG. 8.** Dose-response curve of the vasoconstrictor effect for bethanechol injected into the arterial supply of the inferior mesenteric ganglion before and after atropine.

\* Significant difference from control response to bethanechol (BCH) (p<0.05).

Further explanations as in Fig. 3.
10–20 sec and the response lasted between 1–2 min, depending upon the dose. In the case of large doses of Ach (1000, 2000 µg), the negative chronotropic effects which were maximal 20 sec later and lasted 30 sec followed after tachycardia, probably as a consequence of peripheral action of re-circulated Ach. Blood pressure initially rose after Ach and then fell, the former probably due to the ganglionic effect of Ach and the latter to the peripheral effect. The dose-response curve of the positive chronotropic effect for Ach is shown in Fig. 10. After i.a. administration of C₆ (10 mg/kg) to five spinal dogs in which the mean heart rate was 146 ± 3.9 beats/min, the dose-response curve for Ach was significantly shifted to the right and the maximally attainable response was decreased (the left panel in Fig. 10). The curve was further shifted in a stepwise manner to the right by atropine (0.1 and 0.2 mg/kg) given i.a. in divided doses after C₆ with significant difference from C₆ treatment. An additional treatment with C₆ (10 mg/kg) caused a further shift to the right of the dose-response curve for Ach.

After initial administration of atropine (0.1 mg/kg) which caused a slight and transient decrease in heart rate, the dose-response curve for Ach was shifted to the right but insignificantly with a decrease in the maximally attainable response (the right panel in Fig. 10). C₆ (10 mg/kg) given additionally after atropine shifted the dose-response curve for Ach in a stepwise fashion to the right with decreases in maximal response, and with significant difference from control and atropine treatment. An additional treatment with atropine (0.1 mg/kg) caused a further shift to the right of the dose-response curve for Ach. After these treatments, the rate of positive chronotropic effect of Ach with the high dose of 5000 µg decreased, probably as a result of interference with the peripheral negative chronotropic
FIG. 10. Dose-response curve of the positive chronotropic effects for i.a. acetylcholine and its alteration by hexamethonium and atropine. The left panel shows effect of hexamethonium and additional divided doses of atropine on the response to acetylcholine, the right panel effect of atropine and additional divided doses of hexamethonium on the response. *, Significant difference from control response to acetylcholine; =-, Significant difference from the response after C₆ or atropine alone (p<0.05). Further explanations as in Fig. 3.

DISCUSSION

The preganglionic stimulation of the inferior mesenteric ganglion and i.a. administration of ACh induced a similar frequency- and dose-dependent vasoconstriction of the inferior mesenteric artery. The curves relating to the vasoconstrictor response to the frequency of preganglionic stimulation and to the dose of ACh were shifted to the right by C₆ given i.v. alone or in combination with atropine and by atropine administered i.v. alone or in combination with C₆, except that the curve to preganglionic stimulation was not significantly shifted by atropine administered i.v. alone. These findings with the inferior mesenteric ganglion differ from those previously reported on the cardiac sympathetic ganglia of dog by intravenous administration of antagonists (5). When we gave the antagonists into the subclavian artery, the dose-response curve in positive chronotropic effects of i.a. ACh shifted stepwise to the right by C₆ or atropine. Accordingly, there are both nicotinic and muscarinic...
receptors in the cardiac ganglia. However, Fleisch et al. (5) previously reported that when the antagonists were administered i.v. as done in the present experiment on the inferior mesenteric ganglion, the dose-response curve of positive chronotropic effect for ACh shifted to the left by C6, in contradistinction to the present result with the inferior mesenteric ganglion, while the curve was shifted to the right by atropine. The frequency-response curve of positive chronotropic effect for preganglionic stimulation was shifted to the right by C6 and eliminated by subsequently injected atropine, whereas atropine alone did not alter the curve and the supramaximal doses of subsequent C6 markedly shifted the curve to the right (4). Hilton (7) also demonstrated that the response for cardiac force of contraction induced by close intraarterial injection of ACh was potentiated by chlorisondamine but reduced by atropine. In addition, the response to preganglionic stimulation was depressed by chlorisondamine and further decreased by atropine administered after the nicotinic ganglion blocking agents. It has thus been proposed with the dog cardiac sympathetic ganglia, C6 or chlorisondamine shifts the curve for nerve stimulation to the right but shifts the dose-response curve for i.a. ACh to the left. Atropine, on the other hand, does not affect the curve for nerve stimulation but shifts clearly the dose-response curve for ACh to the right. As a possible explanation for these facts, Fleisch et al. (5) proposed that a localization of muscarinic and nicotinic sites might be different. When the preganglionic nerve is stimulated, ACh may be released from nerve terminals in close proximity to nicotinic receptors, and, after block of these receptors by nicotinic blocking agents, a transmitter action upon more distant muscarinic sites might become manifest. When ACh was administered i.a., access of the agonist to the receptors sites would be from the capillary and extracellular spaces. Accordingly, nicotinic receptors, if hidden in the synaptic cleft, might be less easily accessible, and muscarinic ones, located on neuronal surfaces, more accessible from the vascular space. In the present studies on the inferior mesenteric ganglion, both C6 and atropine inhibited the effects of both preganglionic stimulation and ACh except for insignificant inhibition of responses to stimulation by atropine. In addition, the dose-related vasoconstrictive effect of a pure nicotinic stimulant, TMA, was strongly depressed by C6 i.v. This result is not in keeping with a previous report (6) which stated that in the dog cardiac ganglia, the dose-response curve of positive chronotropic effect for TMA was not strongly inhibited by C6 but did shift to the right. The dose-related vasoconstrictive effect of the muscarinic agonist, bethanechol, was abolished by i.v. administration of atropine, though Herr and Gyermek (8) demonstrated by recording the spike potential of a small number of postganglionic fibers that atropine did not alter the effects of ganglionic stimulants in the inferior mesenteric ganglion of the cat. Therefore, although the present experiment confirms the evidence that the inferior mesenteric ganglion of the dog contains excitatory nicotinic as well as muscarinic mechanisms, the inferior mesenteric ganglion seems to possess a better developed nicotinic system than does the cardiac ganglia, in the dog.

The combined administration of C6 and atropine caused a stepwise shift to the right of the dose-response curve for ACh, but the maximal vasoconstrictive responses to larger doses of ACh were increased. With the cardiac ganglia, a similar phenomenon in the positive
chronotropic effect of ACh was observed by Fleisch et al. (5). These changes may result from the interaction between the two types of receptors located on the neuron, but there has been no plausible explanation for this increase in the maximal effects.

Nicotinic and muscarinic agonists, such as ACh, nicotine, TMA, DMPP and bethanechol, applied to the inferior mesenteric ganglion through the artery, resulted in a vasoconstriction of the inferior mesenteric artery. However, when successive larger doses of the nicotinic agent were administered, the potency of the vasoconstrictive effect was decreased in a dose-dependent manner in the case of larger doses. It was reported that the dose-response curve of the positive chronotropic effect for TMA administered to the dog cardiac ganglia through the artery became similarly bell-shaped, i.e., smaller responses with a large dose (6). By recording continuously both ganglionic surface potential and the activity in a postganglionic nerve in the cat superior cervical ganglion in situ, when the depolarization induced by arterially injected DMPP and other nicotine like drugs exceeded a certain level or was maintained over a certain time, the processes such as inactivation of the Na+ carrying system and increase of depolarization threshold prevented bursts of discharges (9). Therefore, it is probable that these mechanisms are involved in forming a bell-shaped dose-response curve for depolarizing agents such as TMA, DMPP and nicotine with decrease in their own stimulant action with high doses.

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