ACUTE INHIBITORY EFFECTS OF RESERPINE ON THE SYMPATHETICALLY INDUCED RESPONSE OF DOG STOMACH

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It is well known that reserpine causes a depletion of catecholamine and 5-hydroxytryptamine contents in various organs and tissues thereby preventing sympathetic transmission (1, 2). In order to eliminate sympathetic effects on gastro-intestinal motility, reserpine has usually been given to animals for a few days or at least 20 hr before experiment (3-5). One disadvantage of these experiments is that observation of a control response to stimulation of the sympathetic nerve cannot be done.

Recently it has been observed that reserpine added to bathing solution was effective in reducing or abolishing the sympathetically induced response of isolated preparations (6-8). In these experiments, it has been presumed that mechanisms other than an overall depletion of catecholamine contents possibly accounted for the acute inhibitory action of reserpine.

The present experiment was made to elucidate whether or not an acute effect of reserpine can be demonstrated on the sympathetically induced response of the dog stomach in vivo. The result showed that a single large dose of reserpine injected intravenously inhibited selectively the sympathetic nerve response within 4-5 hr.

METHODS

The experiments were performed on adult dogs which had been kept fasting for 24-48 hr. The animals were anesthetized via intravenous injection of pentobarbital sodium 30 mg/kg. Under the anesthesia, the trachea and the femoral artery were cannulated. Polyethylene tubing was also inserted into the cephalic vein for systemic administration of the drug.

A muscle relaxant (gallamine triethiodide, Flaxedil) was injected and immediately artificial ventilation was started. Under these conditions, the left side of the thorax was opened by removing the ribs from 7th to 13th to expose the dorsal vagal trunk and the greater splanchnic nerve. Either the left vagal branch was cut at a position just above its entry into the dorsal trunk, or the dorsal trunk itself was cut, and its peripheral end was separated from the surrounding tissues. The cut end was used for peripheral vagal stimulation. The bilateral greater splanchnic nerves were sectioned at the level of the 13th
The left adrenal gland and spleen were removed after ligations of all blood vessels at points nearest each organ. The perivascular nerve attached to the coeliac artery was separated together with the coeliac ganglion. This nerve was ligated at the caudal pole of the ganglion and its distal side was used for the postganglionic sympathetic nerve stimulation (9). A thin polyethylene tubing was inserted into a branch of the splenic artery for close gastric arterial injection of drugs. A volume of 0.5 ml noradrenaline was injected through this tubing and flushed with 0.5–1 ml of heparinized saline.

In order to record gastric motility, a balloon which had been attached to the end of a polyethylene tube was introduced into the region of the fundus and body of the stomach through the mouth. The balloon was filled with about 100 ml of warm water and was connected to a reservoir mounted on a mechano-electric transducer via the tube. Changes in water volume in the reservoir correspond to the motility of the stomach. Thus as an indicator of the gastric motility, the changes were displayed on an ink writing recorder (Nihon Koden, WA-205) through an electronic manometer (Nihon Koden, MP-4) and direct coupling amplifier (further details, see Ohga et al. 10). The right femoral arterial pressure was also monitored using the electronic manometer.

The vagus and sympathetic nerves were stimulated with an electronic stimulator (Nihon Koden, MSF-3R) through bipolar silver wire electrodes. Stimuli of 1 millisecond duration were applied at a frequency of 20 Hz for 10–30 sec. The voltage was adjusted to be supramaximal in each individual.

Reserpine 5 mg/kg was injected intravenously, after control responses to stimulation of the perivascular and vagus nerves, and injection of noradrenaline into the gastric artery had been recorded, respectively. After administration of reserpine, these responses were observed for 6 or 7 hr.

Drugs used were dl-noradrenaline hydrochloride (Sankyo), atropine sulphate (Merck), bethanechol chloride (Eizai), gallamine triethiodide (Flaxedil, Specia), phenoxybenzamine hydrochloride (Tokyo-kasei) and reserpine (Serpasil, CIBA-Geigy).

RESULTS

The effect of reserpine on responses of the stomach to stimulation of the perivascular nerve (sympathetic nerve) was observed at 1 hr intervals after intravenous injection of 5 mg/kg. In order to avoid contribution of the cholinergic excitatory effect to gastric responses, the animals were administered atropine repeatedly throughout the observation.

In confirmation of the results of our previous paper, the responses to sympathetic nerve stimulation of the stomach of the atropinized dog were variable, namely, a contraction, a contraction followed by a relaxation and a relaxation. Furthermore, there were some cases in which little change in the tone of the stomach was produced, even if the stimulus intensity was increased. The response to close arterial injection of noradrenaline (5–10 μg) was usually consistent with the sympathetically induced response (9). On the other hand, response to vagal nerve stimulation was always a relaxation which was assumed to be nonadrenergic in origin (10, 11).
Fig. 1. Effects of reserpine on the gastric responses to stimulation of the perivascular (Peri. S) and vagus (VPS) nerves and to close arterial injection of noradrenaline (NA) 10 μg in the atropinized dogs. A, control response, B, 5 hr after intravenous injection of reserpine 5 mg/kg, C, 10 min after intravenous infusion of noradrenaline 0.5 mg/15 min. The traces from above downwards are the time marker, gastric response and blood pressure. Stimulation for 10 or 30 sec and close arterial injection of noradrenaline are indicated as the half blank on the time trace and marks and sequences are illustrated above the control panel. Calibration is given on the lowest panel, except that of the blood pressure on the control trace in this figure. The same is applied to the following figures unless otherwise indicated.

In the present experiments, responses to vagal stimulation and to noradrenaline injection served as a convenient control for determining the selective effect of reserpine on the sympathetically induced response.

Effect of reserpine in the atropinized dog

This series of experiments was performed on seven dogs. The response to stimulation of the perivascular nerve was either a contraction followed by a relaxation, or a straight contraction. Reserpine markedly reduced or abolished the sympathetic response 4–5 hr after intravenous injection (Fig. 1). On the other hand, the drug did not significantly affect the responses caused by vagal stimulation and by noradrenaline injection. Pressor response to stimulation of the perivascular nerve was also decreased by reserpine, but the decrement was smaller than that of the gastric response.
Neither the reduced sympathetic response of the stomach nor the pressor response were restored by intravenous infusion of noradrenaline 0.5-1 mg dissolved in 50 ml saline for 15 min (Fig. 1).

In four out of seven dogs, a tonic contraction was produced by sympathetic stimulation after the typical sympathetic response was abolished by reserpine. The tonic contraction was resistant to phenoxybenzamine whereas sympathetically induced contractions before treatment with reserpine were easily abolished (Fig. 2). In this figure, it is noted that contraction caused by noradrenaline is reversed by phenoxybenzamine to a relaxation, while the tonic contraction is not.

**Effect of reserpine in the dog pretreated with atropine plus phenoxybenzamine**

Pretreatment with phenoxybenzamine

It has been shown that various responses of the stomach to sympathetic nerve stimulation are converted into a pure relaxation after administration of phenoxybenzamine in the atropinized dog (9). For this reason phenoxybenzamine was used for easy observation of the effect of reserpine on the sympathetically induced relaxing response.

Phenoxybenzamine is an α-blocker, but, on the other hand, it is also an inhibitor to the uptake of noradrenaline in isolated tissues (12-15). Moreover, this drug causes an increase in the outflow of noradrenaline by stimulation of the sympathetic nerve (16-18). Thus phenoxybenzamine may accelerate the lowering of the noradrenaline content in the nerve endings and eventually cause exhaustion of the transmitter (13, 19, 20).

For this reason, a test was first carried out to see whether or not the relaxing response to stimulation of the sympathetic nerve is reduced by phenoxybenzamine. As a result,

![Fig. 2. Tonic contraction caused by stimulation of the perivascular nerve after treatment with reserpine in the atropinized dogs.](image)

A, responses 4 hr and B, 6 hr after injection of reserpine. Between A and B, phenoxybenzamine 15 mg/kg is injected intravenously.
FIG. 3. Persistence of the relaxing responses after treatment with phenoxybenzamine in the atropinized dogs. A, relaxing responses 1 hr (a) and 6 hr (b) after injection of phenoxybenzamine, B, plotting of the amplitude of each relaxation. Each point represents the mean of five dogs, o-o, stimulation of perivascular and x-x, vagus nerves, ●-●, close arterial injection of noradrenaline 10 μg. Ordinate: amplitude of relaxation expressed as a percentage of the control response, Abscissa: time after phenoxybenzamine injection.

the relaxation caused by stimulation of the sympathetic nerve hardly changed for more than 6 hr after intravenous injection of phenoxybenzamine 15 mg/kg (Fig. 3A). Both responses to vagal stimulation and noradrenaline were also unaffected. The mean amplitude of these responses derived from five dogs are shown in Fig. 3B, plotting the percentage of the control response against the time lapse after the phenoxybenzamine injection.

Effect of reserpine on the relaxing response

The effect of reserpine on the relaxing response to sympathetic nerve stimulation was observed using thirteen dogs which had been pretreated with atropine plus phenoxybenzamine. Reserpine 5 mg/kg was injected intravenously at least one hr after administration of phenoxybenzamine. In all dogs, reserpine markedly reduced or abolished the sympathetically induced relaxation 4 or 5 hr after injection (Fig. 4). The time course of the inhibitory effect is graphically shown in Fig. 5. Reduction of the relaxation was about 60% in 2 hr and 85% in 5 hr.

In five out of thirteen dogs, a tonic contraction which was very similar to that obtained in the atropinized dogs appeared after treatment with reserpine as shown in Fig. 2B.
FIG. 4. Effect of reserpine in the dog pretreated with atropine plus phenoxybenzamine.
A, control response, B, 2 hr, C, 3 hr and D, 5 hr after injection of reserpine.

FIG. 5. Time course of the effect of reserpine. o-o, stimulation of perivascular and x-x, vagus nerves, ● ●, close arterial injection of noradrenaline 10 μg. Each point represents the mean amplitude of relaxation of eight dogs. The vertical bar indicates the standard errors of the means. Ordinate; amplitude of relaxation expressed as a percentage of the control response, Abscissa; time after reserpine injection.

Usually, the tonic contraction was preceded by a small relaxation. Additional administration of atropine and phenoxybenzamine did not affect the contraction. Responses to vagal stimulation and to noradrenaline were always relaxation throughout the time course of the experiments.

DISCUSSION

The present results show that reserpine injected intravenously can reduce or abolish the sympathetically induced responses of dog stomach within 4 or 5 hr. This acute effect of reserpine was obtained without affecting the relaxing response to vagal stimulation, which was assumed to be nonadrenergic in origin (10, 11, 21, 22), and the response to close arterial injection of noradrenaline. In addition, reserpine reduced or abolished the relaxing response to sympathetic nerve stimulation in dogs pretreated with phenoxybenzamine. As mentioned briefly in the results, it has been reported that phenoxybenzamine inhibits the uptake of noradrenaline in the sympathetic nerve ending and causes an exhaustion of the transmitter in various isolated organs. In the present experiments, however, reduction of the sympathetically induced relaxation for over 6 hr after administration of only phenoxybenzamine was not observed. On the basis of these results, it is concluded that the acute
inhibitory effect of reserpine is due to its selective action on the endings of the sympathetic nerve.

In reserpinized dogs, intravenous infusion of noradrenaline failed to restore the effect of sympathetic nerve stimulation on the stomach and blood pressure. It is reported that reserpine does not prevent the uptake of exogenous noradrenaline by tissues, but impairs the process involved in the intraneuronal storage of this amine. Thus, even if exogenous noradrenaline is taken up by sympathetic nerve tissues, it cannot be retained for a long time (23). Toda et al. (24), however, reported that the chronotropic effect of sympathetic nerve stimulation of the isolated atria from a reserpinized rabbit was not restored by adding either noradrenaline or dopamine, but the effect of tyramine was restored. Accordingly, it seems possible that reserpine, especially in the acute phase, can also act through an unknown mechanism in addition to that already well-known in the depletion of the transmitter.

In some isolated preparations, it has been suggested that an acute inhibitory effect of reserpine on the sympathetically induced response may be derived from mechanisms other than an overall depletion of catecholamine. Day and Warren (6) observed that reserpine added to the bathing solution abolished both responses of the isolated rabbit ileum to sympathetic nerve stimulation and to transmural stimulation. The abolished response to sympathetic nerve stimulation was restored after addition of dopamine to the medium, but not the response to transmural stimulation. From these results, they assumed that reserpine could exert its acute effect also through a different action from its catecholamine depleting action. Euler (7) observed in the isolated guinea pig vas deferens that reserpine also rapidly inhibited the response to hypogastric nerve stimulation. This effect was considered to be caused by a local deficiency of the transmitter immediately available for release, rather than by an overall depletion of the transmitter contents. Moreover, Kubo et al. (8) showed that the neuromuscular transmission of the isolated perfused rabbit heart partially failed through perfusing of the reserpine solution and that this was consistent with a decrease in the noradrenaline output released by sympathetic nerve stimulation. They attributed the main mechanisms for the decrease in the transmitter output to a stabilizing action of reserpine on the neural membrane. In the present experiments, however, vagally induced relaxation was not affected by reserpine and the stabilizing action of this drug on the neural membrane could not be expected. In any case, the drug appears to be useful as a selective adrenergic neuron blocking agent like bretylium or guanethidine.

Previously we reported that the contraction of the stomach caused by sympathetic nerve stimulation was mediated through the activation of the cholinergic nerve and adrenergic α-receptor in the dogs (9). In some cases in the present experiment, another long-lasting contraction was seen with stimulation of the sympathetic nerve in the reserpinized dogs after the usual sympathetic response had been greatly reduced. This contraction did not appear until the animal was given reserpine. Arterial injection of noradrenaline never produced the same contraction. In a few previous experiments, a similar contraction was also observed after injection of guanethidine or bretylium. The long-lasting contraction was not abolished by additional administration of atropine and phenoxybenzamine. It
appears likely that the adrenergic neuron blockade results in the unmasking of an excitatory mechanism other than either the cholinergic or adrenergic mechanisms in the sympathetic pathways to the stomach.

**SUMMARY**

Acute effects of reserpine on the response of the stomach to perivascular nerve stimulation were studied in dogs, anesthetized with pentobarbital sodium 30 mg/kg i.v. The animals were pretreated with either atropine or atropine plus phenoxybenzamine. Reserpine 5 mg/kg i.v. reduced or abolished the sympathetically induced responses after 4-5 hr. The rate of the inhibition was approx 60%, 2 hr after injection, and 85%, 5 hr after injection, of reserpine. The relaxation caused by vagal stimulation and by noradrenaline was not significantly affected by reserpine. It can be concluded that a single large dose of reserpine can selectively inhibit the sympathetically induced response of the stomach within a relatively short period. Site of the action appears to be in the presynaptic endings of the postganglionic sympathetic nerves. In some cases, stimulation of the sympathetic nerve caused a longlasting contraction after a typical sympathetic response was inhibited by reserpine.

**REFERENCES**

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