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A periarterial nerve-circular muscle preparation from the caecum of the guinea-pig

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A periarterial nerve-circular muscle preparation from the caecum of the guinea-pig has been described. Stimulation of the periarterial nerve produced relaxation of the circular muscle strip. These responses were blocked by adrenergic neurone blocking agents but not by ganglion blocking drugs. Phenoxybenzamine or piperoxan greatly reduced the relaxation but propranolol abolished it. No relaxation was observed on preparations from animals pre-treated with reserpine. It was observed that after the blockade of the responses to periarterial nerve by adrenergic neurone blocking drugs, stimulation of the periarterial nerves in the presence of physostigmine induced contractions which were blocked by local anaesthetics or hyoscine but not consistently by ganglion blocking drugs.

IN 1930, Finkleman described the effect of periarterial nerve stimulation on the isolated intestine of the rabbit. He recorded the responses of the longitudinal muscle and obtained relaxation and less frequently contractions. Since that time, many workers have examined the nature of the responses and also the effect of drugs on these responses (Day & Rand, 1961; Gillespie & MacKenna, 1961; Bentley, 1962; Boyd, Gillespie & MacKenna, 1962). The effect of the periarterial nerve stimulation on the circular muscle does not seem to have been examined in detail. Garry & Gillespie (1955) reported that the stimulation of the sympathetic nerve to the rabbit colon inhibited both muscle coats. Van Harn (1963) obtained contraction or relaxation of the circular muscle of the cat intestine on stimulation of the sympathetic nerve.

A periarterial nerve-circular muscle preparation of the guinea-pig caecum is described and the nature of the responses to the nerve stimulation has been investigated.

Methods

Guinea-pigs weighing about 500 g were killed by stunning and bleeding. The preparation of the perivascular nerve-caecal circular muscle strip is described in Fig. 1.

The periarterial nerve was stimulated with supramaximal voltage (about 50 V) at a frequency of 50 shocks/sec and at a pulse duration of 0·1 msec unless otherwise stated. The stimulation was for 20 sec every 4 min and this timing was automatically controlled with an 'Interval Timer' (Electrical Remote Control Co. Ltd.) The responses were magnified 10 times and were recorded on smoked paper with an isotonic frontal-writing lever.

Drugs. The drugs used were bethanidine sulphate, bretylium tosylate, dexamphetamine sulphate, dimethylphenylpiperazinium iodide, guanethidine sulphate, hexamethonium bromide, hyoscine hydrobromide, (–)-noradrenaline bitartrate, pentolinium tartrate, phenoxybenzamine

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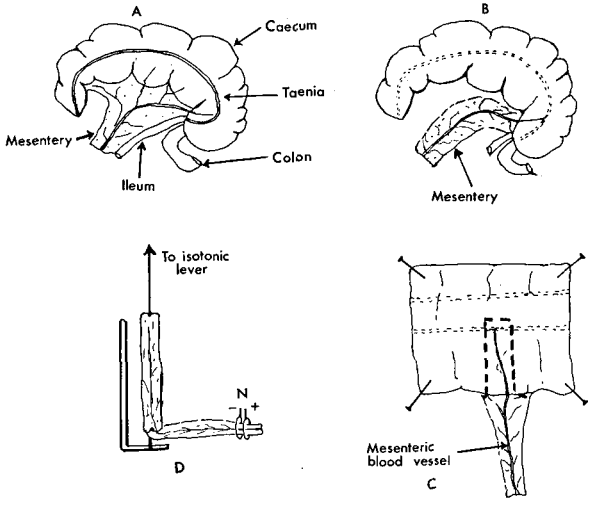

FIG. 1. The preparation of the perivascular nerve—caecal circular muscle strip of the guinea-pig. The upper drawing on the left (A) shows the caecum with parts of the ileum, the colon and the mesentery with its blood vessels. One of the taeniae is also shown. The taenia caeci distant to the mesentery were dissected and the ileum separated from the mesentery. The mesentery with its blood vessels was freed from the end of the caecum distal to the ileo-caecal junction to a point about 1 cm from the junction (B). The caecum was then cut open along its length near the mesenteric border so that the mesentery remained attached to the opened caecum. It was washed in Krebs solution and pinned out with the mucosal surface downwards on a cork pad in the Krebs solution. The drawing "C" shows a part of the caecal wall with the mesentery. One of the blood vessels running from the mesentery across the caecal wall is shown. The caecal circular strip 4 cm long by 4 mm wide was cut to include this blood vessel as shown in "C". The end of the strip near the mesentery was tied to a holder. The preparation was then suspended in an organ bath containing 80 ml Krebs solution at 37° gassed with 95% oxygen 5% carbon dioxide. The other end of the strip is attached to an isotonic lever (D). The mesentery with its blood vessels was pulled through two platinum ring electrodes (Birmingham & Wilson, 1963) for the perivascular nerve stimulation (N).

hydrochloride, physostigmine salicylate, piperoxan hydrochloride, propranolol hydrochloride and reserpine. The concentrations of all the drugs are expressed as final bath concentrations in μg/ml of the base.

Results

Stimulation of the periarterial nerve to the caecal circular muscle strip at a pulse duration of 0·1 sec produced inhibition of the preparation. The response increased with the frequency of stimulation from 12 shocks/sec and was maximal at about 50 shocks/sec. It also increased with pulse duration from 0·01 msec to reach a maximum at 0·3 msec. These responses were not obtained when the mesentery between the electrodes and the circular muscle was cut.

The action of ganglion blocking drugs. The inhibitory responses to periarterial nerve stimulation were not modified by hexamethonium or pentolinium in concentrations up to 100 μg/ml.

The effect of α- or β-adrenergic receptor blocking agents. Fig. 2 illustrates the typical effect of phenoxybenzamine or propranolol on the responses to perivascular nerve stimulation. Phenoxybenzamine (0·1 μg/ml) greatly reduced the responses but a higher concentration (5 μg/ml) did not abolish them. The relaxation was reduced by propranolol (5 μg/ml) and eliminated by 10 μg/ml of propranolol. Piperoxan (5–10 μg/ml) reduced but did not abolish the responses.

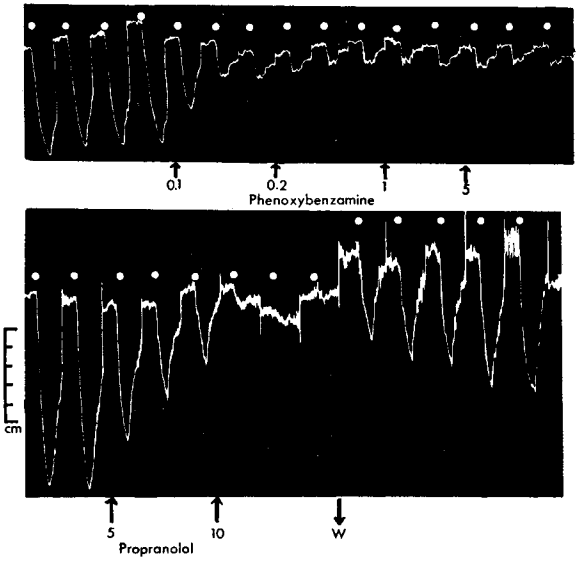

FIG. 2. The effect of phenoxybenzamine and propranolol on the responses of the caecal circular muscle to perivascular nerve stimulation. The preparations were stimulated for 20 sec every 4 min at a frequency of 50 shocks/sec, with a pulse duration of 0·1 msec and supramaximal voltage. The numbers represent the concentration of the drugs in μg/ml of the bath fluid. Phenoxybenzamine (0·1 μg/ml) greatly reduced the relaxations but increasing the concentration to 0·2, 1 or 5 μg/ml did not abolish the responses (upper tracing). Propranolol (5 μg/ml) greatly reduced the responses and a higher concentration (10 μg/ml) abolished them. When the preparation was washed, the responses partly returned.

The influence of adrenergic neurone blocking drugs. Guanethidine (2-8 μg/ml) blocked the responses to periarterial nerve stimulation. This blockade persisted after washing out the antagonist drug and was only slightly reversed by the addition of dexamphetamine (1–10 μg/ml). The inhibitory responses always returned when dexamphetamine was washed out of the bath. When dexamphetamine (2 μg/ml) was present in the bath, guanethidine (2 μg/ml) did not modify the responses of the circular muscle to periarterial nerve stimulation (Fig. 3).

Blockade of the responses of the circular muscle strips was produced with bretylium (4–8 μg/ml), bethanidine (3 μg/ml) or dimethylphenylpiperazinium (5 μg/ml). The effects were partially reversed by dexamphetamine (1–10 μg/ml) but were completely reversed when dexamphetamine was washed out.

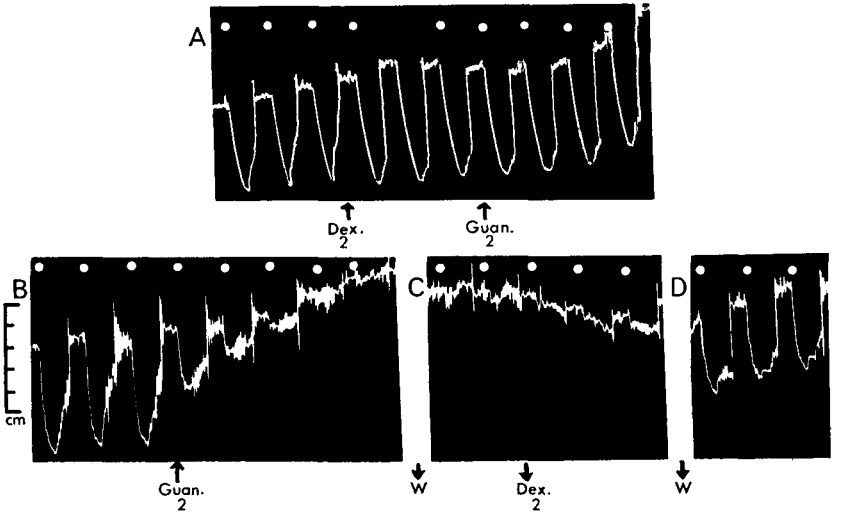

FIG. 3. The effect of guanethidine (Guan) and dexamphetamine (Dex) on the responses of the caecal circular muscle to perivascular nerve stimulation. The parameters and the intervals of stimulation were the same as in Fig. 2. The upper record shows the effect of guanethidine (2 μg/ml) on the responses of circular muscle in the presence of dexamphetamine (2 μg/ml). Dexamphetamine slightly enhanced the relaxations and guanethidine did not modify the relaxations. The lower records show the effect of guanethidine (2 μg/ml) on the responses. The relaxations were slowly abolished. The responses did not return when the preparation was washed (between B and C). The responses only slightly returned in the presence of dexamphetamine (2 μg/ml) in "C", but after washing out the dexamphetamine, complete recovery of the responses was obtained (D).

The effect of pre-treatment with reserpine. Four guinea-pigs were treated with reserpine, 5 mg/kg daily for two days by intraperitoneal injection, and killed on the third day. Little or no response to periarterial nerve stimulation was elicited in any of the preparations but noradrenaline produced inhibition of these circular muscle strips.

Contractions to periarterial nerve stimulation. It was observed that after washing out the adrenergic neurone blocking agent, stimulation of the periarterial nerve in the presence of physostigmine (0·1 μg/ml) induced contractions of the preparation. The contractions were usually small and were not increased by increasing the voltage to 100 V or reducing the frequency to 6 or 12 shocks/sec. These contractile responses were reduced by hexamethonium (100 μg/ml) in two experiments or by pentolinium (50–100 μg/ml) in two out of four experiments. Dimethylphenylpiperazinium (5 μg/ml) blocked the contractions in two experiments but only reduced them in six others. The contractions were blocked by cocaine (30 μg/ml) or hyoscine (0·1 μg/ml) or by cutting the mesentery between the electrodes and the circular muscle. The effect of pentolinium or hyoscine is shown in Fig. 4.

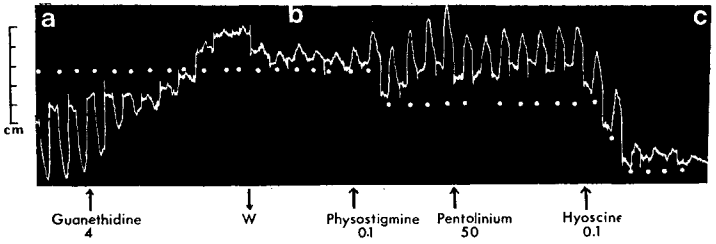

FIG. 4. The contractions of the caecal circular muscle preparation to perivascular nerve stimulation. The perivascular nerve was stimulated with a pulse duration of 0·1 msec and supramaximal voltage. Between "a" and "b" the frequency of stimulation was 5 shocks/sec and between "b" and "c" the frequency was 12 shocks/sec. The intervals of stimulation was as in Fig. 2. The numbers represent the doses of the drugs in μg/ml of the bath fluid. Guanethidine (4 μg/ml) slowly abolished the relaxations to perivascular nerve stimulation. When the preparation was washed at W, very small contractions to perivascular nerve stimulations were obtained. Note that the contractions did not increase when the frequency of stimulation was reduced to 12 shocks/sec. In the presence of physostigmine (0·1 μg/ml), the contractions were enhanced. These contractile responses were only reduced by pentolinium (50 μg/ml) but were abolished by hyoscine (0·1 μg/ml).

Discussion

Stimulation of the periarterial nerve to the caecal circular muscle produced relaxation of the preparation. These inhibitory responses were blocked by adrenergic neurone blocking drugs. Day (1962) reported that dexamphetamine prevented or reversed the effect of adrenergic neurone blocking drugs on the responses to sympathetic nerve stimulation. In the present experiments, dexamphetamine prevented the effect of these blocking drugs but reversed their effects only after it was washed out of the bath.

The inhibitory responses were almost abolished by phenoxybenzamine or piperoxan. Propranolol blocked the responses. This is indicative of catecholamine involvement in the response. However, propranolol is known to have local anaesthetic activity (Morales-Aguilera & Vaughan Williams, 1965) and this perhaps played a part in its blocking action.

Reserpine pre-treatment eliminated the relaxation to the periarterial nerve stimulation. This again provided additional evidence for the adrenergic nature of the response. Gillespie & MacKenna (1961), and Bentley (1962) reported that reserpine pre-treatment not only prevented the relaxation of the longitudinal muscle of the rabbit gut to sympathetic nerve stimulation, but also converted the responses to contractions. No contractions were observed on the circular muscle strips.

The responses of the caecal circular muscle to periarterial nerve stimulation resembled in many respects those of the longitudinal muscle. Finkleman (1930) found that the inhibition of the rabbit intestinal segments was antagonised by ephedrine. When adrenergic neurone blocking drugs became available it was shown, as in the present experiments, that the responses were blocked by these agents (Boura & Green, 1959, 1963; Maxwell, Plummer, Schneider, Povalski & Daniel, 1960; Bentley, 1962; Wilson, 1962; Birmingham & Wilson, 1965).

Garry & Gillespie (1955) stated that stimulation of the lumbar sympathetic nerve to the rabbit colon caused inhibition of the circular muscle. A similar observation has been made on the cat gut (Van Harn, 1962). The present experiments provided evidence for the adrenergic innervation of the caecal circular muscle. Probably other intestinal circular muscles are similarly innervated. Norberg (1964) found that most adrenergic nerves within the gut wall terminated in the plexuses. It is likely therefore that the transmitter released on stimulation of the periarterial nerve diffuses to the circular muscle cells to activate the receptors.

It has been found that adrenergic neurone blocking drugs converted the responses of the intestinal longitudinal muscle to sympathetic nerve stimulation from inhibition to contraction (Day & Rand, 1961; Bentley, 1962; Boyd & others, 1962). A similar observation has now been made on the circular muscle. Day & Rand (1961) found that the contractions of the rabbit ileum to periarterial nerve stimulation revealed by guanethidine were not always blocked by hexamethonium. They suggested that their results represented a cholinergic link in the sympathetic mechanism according to the hypothesis of Burn & Rand (1959). The contractions of the rabbit gut to sympathetic nerve stimulation produced by guanethidine or after pre-treatment with reserpine were shown by Bentley (1962) to be blocked by hexamethonium. Bentley favoured the suggestion of Gillespie & MacKenna (1961) that the contractions were due to stimulation of the preganglionic parasympathetic fibres. The results obtained on the circular muscle did not disprove or confirm either of the above possibilities. The inconsistency of the results with ganglion blocking drugs made the interpretation difficult. Probably the nerves were cholinergic since the responses were blocked by hyoscine.

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