A periarterial nerve-longitudinal muscle (taenia) preparation from the guinea-pig caecum

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The isolated taenia from the guinea-pig caecum has been used extensively for physiological and pharmacological studies (see Burnstock, 1972). Burnstock, Campbell & Rand (1966) described a perivascular nerve-taenia preparation from the caecum of the guinea-pig and used the preparation to study the inhibitory innervation of the taenia. This preparation still has the circular muscle attached to the taenia.

An isolated taenia preparation free of the circular muscle but with the periarterial nerve attached is described and the responses to stimulation of the nerve have been investigated. It is possible with this preparation to record responses to periarterial nerve stimulation and those to stimulation of non-adrenergic inhibitory nerves due to transmural stimulation, alternately. The preparation seems most suitable for investigating the mechanism of the inhibitory responses of the taenia to the stimulation of the extrinsic and intrinsic nerves, as the responses will not be modified by the presence of the circular muscle.

Guinea-pigs weighing about 450 g were killed by stunning and bleeding. The abdomen was opened, and mesentery lying between the caecum and the terminal part of the ileum was exposed. The mesentery was separated from the ileum. The taenia lying close to the mesentery (in the caecal curvature) was carefully dissected from the rest of the caecum such that the mesenteric attachment to the taenia remained intact (Fig. 1). The length of the taenia from the distal end of the caecum to a point about 3 cm from the ileo-caecal junction was used.

The preparation was set up in Krebs solution at 32°. One end of the taenia was tied to a holder and the other to a frontal writing lever. The mesentery with its blood vessels was pulled through two unshielded platinum ring electrodes (about 4 mm apart) mounted on a Perspex holder. With the exception of the rings, the rest of the platinum wires were cemented into grooves in the Perspex frame.

For transmural electrical stimulation, the preparation was arranged so that the taenia was suspended between two parallel platinum electrodes about 4 mm apart fixed on either side of a Perspex channel as described by Birmingham & Wilson (1963) (Fig. 1D). With this set up, it was possible to record responses to transmural and to periarterial nerve stimulation alternately from the same preparation.

Stimulation of the periarterial nerve at 0·3 ms, and supramaximal voltage produced relaxation of the taenia. This relaxation increased with frequency of stimulation from 6 Hz to reach a maximum at a frequency of 50 or 100 Hz. The inhibitory responses also increased with an increase in pulse duration from 0·03 ms to reach a maximum at about 0·3 ms (6 experiments). In all the experiments reported below, the periarterial nerve was stimulated at 0·1 ms, 50 Hz and supramaximal voltage. A pulse duration of 0·3 ms at a frequency of 50 Hz was not used because of the big changes in the tone of the tissue which it produced (5 experiments).

The typical response to the periarterial nerve stimulation consisted of a relaxation which reached maximum in about 15 s. At the end of the stimulation period the preparation recovered slowly and often contracted slightly before finally attaining its original length. All the effects of stimulating the periarterial nerve were abolished by cutting the mesentery between the electrodes and the taenia.

Transmural stimulation at 0·3 ms and at frequencies above 0·5 Hz and supramaximal voltage, induced contraction or relaxation or a biphasic response. The

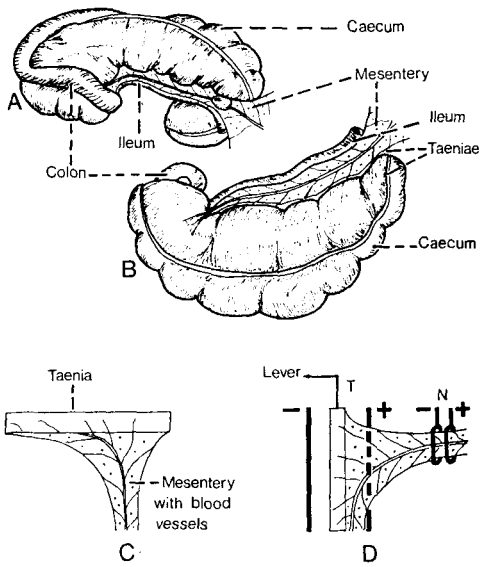

FIG. 1. Preparation of the periarterial nerve-taenia preparation from the guinea-pig caecum. The upper drawing 'A' shows the caecum with parts of the colon and ileum. Two taeniae, one of which lies near the mesentery are shown in 'B'. The ileum is separated from the mesentery and the taenia lying close to the mesentery is dissected so that the mesentery with its blood vessels is still attached to it, 'C'. In 'D' the taenia is shown set between two parallel platinum electrodes for transmural stimulation (T). The mesentery is pulled through two platinum ring electrodes for periarterial nerve stimulation (N).

contractions increased with frequency to reach a maximum at about 50 Hz. In some preparations, stimulation at low frequency (12 Hz or less) produced a small contraction followed by a relaxation. In such preparations, when the stimulation was stopped, a contractile response was observed. This type of contraction which occurred at the end of the stimulation will be called 'after-contraction'. When the frequency was increased to 25 or 50 Hz, a contraction was obtained but this contraction was followed by an immediate relaxation at the end of the stimulation period. This type of delayed response will be referred to as the 'after-relaxation'. Transmural stimulation of the taenia at times produced a short-lived relaxation followed by contraction.

The only factor which seemed to determine the type of response obtained during transmural stimulation was the degree of tone exhibited by the taenia. In preparations which developed little or no tone, transmural stimulation produced contraction at all frequencies. When a high degree of tone was present, transmural stimulation at low frequencies (1–12 Hz) usually produced relaxation and that at high frequencies induced contractions or biphasic response.

In the presence of hyoscine (0·1 μg ml^{-1}), transmural stimulation of the taenia caused an immediate relaxation and on cessation of the stimulation, an 'after-contraction' was seen. The preparation then slowly relaxed. The relaxation increased sharply as the frequency was increased from 0·5 Hz reaching a maximum at about 12 Hz. The 'after-contraction' also increased as the frequency was raised. The relaxations increased with changes in pulse duration from 0·1 ms to reach a maximum at about 1·0 ms and then decreased at higher pulse durations. In all the experiments reported below, the transmural stimulation in the

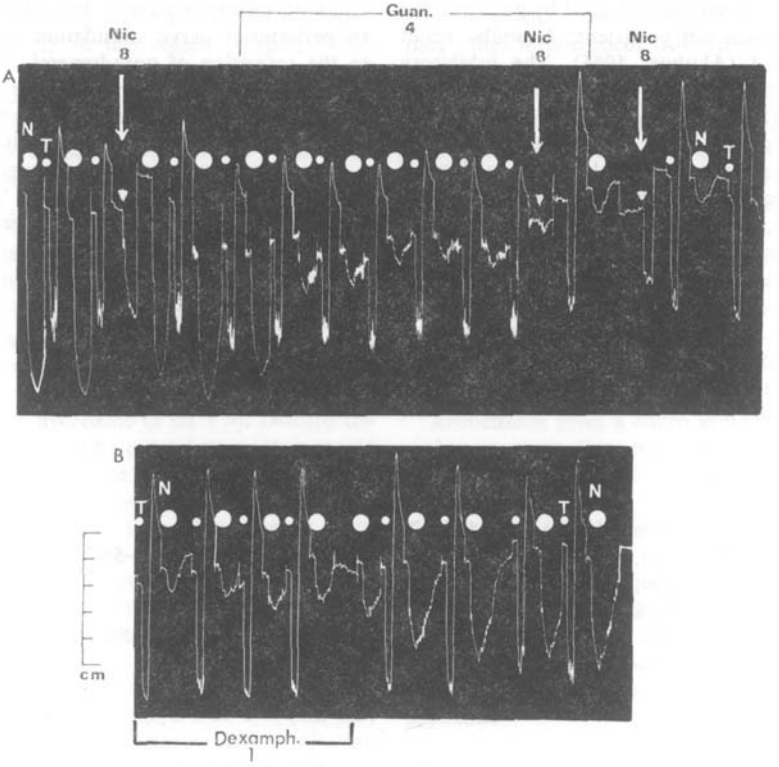

FIG. 2. A kymograph record of the inhibitory responses of the taenia, in the presence of hyoscine (0·1 μg ml^{-1}), induced alternately by periarterial nerve stimulation (N) and by transmural stimulation (T). The periarterial nerve stimulation was at 50 Hz, 0·1 ms and supramaximal voltage while the transmural stimulation was 12 Hz, 0·3 ms and supramaximal voltage. Each stimulation was for 20 s and the kymograph was allowed to run for an extra 10 s after stopping the stimulation. The interval between the stimulations was 5 min. Inhibitory responses to nicotine (Nic) 8 μg ml^{-1} are also shown,

In panel A, guanethidine (Guan) 4 μg ml^{-1} blocked the responses to periarterial nerve stimulation and to nicotine but not those to transmural stimulation. On washing out the guanethidine from the bath, the responses to periarterial nerve stimulation were still blocked but the response to nicotine returned.

In panel B, when (+)-amphetamine (Dexamph) 1 μg ml^{-1} was added to the bath fluid, the relaxation to periarterial nerve stimulation slightly returned but when (+)-amphetamine was washed out, the relaxations slowly returned.

presence of hyoscine (0·1 μg ml^{-1}) was at 0·3 ms, 12 Hz and supramaximal voltage (90 V), while the periarterial nerve stimulation was at 0·1 ms, 50 Hz and supramaximal voltage (50 V). The transmural and the periarterial nerves stimulations were alternated and the interval between them was 5 min. Nicotine which was used in the study for comparison, always produced relaxation of the taenia in the presence of hyoscine (see Akubue, 1966).

The inhibitory responses of the taenia to transmural or to periarterial nerve stimulation were blocked by tetrodotoxin (0·1 μg ml^{-1}). The relaxation produced by nicotine was blocked by the same concentration of tetrodotoxin.

Guanethidine (4–8 μg ml^{-1}) in 10 experiments consistently blocked the inhibitory responses to periarterial nerve stimulation. This blockade persisted after washing out the guanethidine from the bathing fluid but was reversed by (+)-amphetamine (1 μg ml^{-1}). The relaxation to nicotine was blocked by guanethidine but the blockade was not persistent. A similar result has been reported (Akubue, 1966). The inhibitory responses to transmural stimulation were not modified by guanethidine (Fig. 2).

It has been shown that (+)-amphetamine can prevent the adrenergic neuron blocking action of guanethidine (Day, 1962). In the presence of (+)-amphetamine (1μg ml^{-1}), guanethidine (4 μg ml^{-1}) did not block the responses of the taenia to periarterial nerve stimulation but it continued to antagonize the inhibitory responses of the preparation to nicotine.

Results similar to those recorded with guanethidine were also obtained with bethanidine (3 μg ml^{-1}) and dimethylphenylpiperazinium (8 μg ml^{-1}) (see Birmingham & Wilson, 1965).

Pentolinium (50–100 μg ml^{-1}) did not modify the responses of the taenia to transmural or to periarterial nerve stimulation, but the effects of nicotine were blocked by 20 μg ml^{-1} pentolinium (6 experiments).

Phenoxybenzamine (0·5 μg ml^{-1}) or propranolol (5 μg ml^{-1}) or a combination of the two, reduced the relaxations to periarterial nerve stimulation or to nicotine but did not modify the responses to transmural stimulation (5 experiments). Higher concentrations of these blockers (5 and 10 μg ml^{-1} respectively) reduced the tone of the tissue and made the interpretation of their effects difficult.

Pretreatment of guinea-pigs (8) with reserpine 5 mg kg^{-1} (i.p.) daily for two days, greatly reduced the relaxation to periarterial nerve stimulation but the extent of reduction was variable. There was no evidence that this treatment modified the responses to transmural stimulation or to nicotine.

The results obtained with the various antagonists and with reserpine seem to indicate that the responses to periarterial nerve stimulation were due probably to the activation of noradrenergic nerves while those to transmural stimulation resulted from the stimulation of non-adrenergic nerves. The mechanism of action of nicotine on the preparation needs further analysis.

The responses seen after stopping the electrical stimulation ('after-relaxation' and 'after-contraction') were difficult to analyse. The 'after-relaxations' were not obtained in the presence of hyoscine. The 'after-contractions' were reduced by blockers which also reduced the tone of the preparation. Changes in tone also modified the responses.

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