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THE MECHANISM OF THE MOTOR RESPONSE TO PERIARTERIAL NERVE STIMULATION IN THE SMALL INTESTINE OF THE RABBIT

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In the presence of guanethidine, periarterial nerve stimulation caused a frequency-dependent motor response in the rabbit isolated ileum. Tachyphylaxis to capsaicin greatly diminished the effect of periarterial stimulation; the remaining portion of the contractile response was susceptible to hexamethonium. The motor effect of periarterial stimulation is mediated by two different mechanisms: one via parasympathetic preganglionic nerves and the other sensitive to capsaicin.

Introduction Periarterial nerve stimulation elicits a cholinergically-mediated contraction in the rabbit intestine *in vitro* in new-born animals (Burn, 1968), at low frequencies of stimulation (Finkleman, 1930), or following sympathetic blockade (Day & Rand, 1961; Gillespie & Mackenna, 1961; Bentley, 1962; Boyd, Gillespie & Mackenna, 1962). This motor response has been attributed to stimulation of admixed parasympathetic fibres (Gillespie & Mackenna, 1961; Bentley, 1962; Boyd *et al.*, 1962), or taken as an indication of the release of acetylcholine from sympathetic adrenergic nerve endings (Day & Rand, 1961; Burn, 1968; Burnstock, 1978).

In this paper, we show that the motor response of the rabbit ileum, *in vitro*, to periarterial stimulation consists of two, apparently independent, components, one prevented by capsaicin, a sensory neurone-blocking drug (see Szolcsányi & Barthó, 1978), the other by the ganglionic blocking drug, hexamethonium.

Methods Albino rabbits, 12 to 20 days old, from six litters were used. At this age the motor response of the small intestine to periarterial stimulation is more prominent than in the adult and adrenergic responses may also be obtained (cf. Day & Rand, 1961). Ileal segments were set up according to Finkleman (1930) in Krebs solution at 37°C, gassed with 5% CO_2 in O_2. Contractions were recorded isotonically. For quantitative evaluations the half size of the pendulum movements was taken as the base line. Periarterial (10 V, 1 ms) or field stimulation (40 V, 0.1 ms) was applied through pairs of platinum ring or longitudinal electrodes, respectively.

Results When first applied, capsaicin (Calbiochem) caused a dose-dependent motor response of the ileum. The magnitude of the responses evoked by capsaicin 3.3×10^{-6}, 10^{-5} and 3.3×10^{-5} M corresponded to 4.0 ± 2.2 ($n = 5$), 13.0 ± 1.3 ($n = 8$) and 18.8 ± 6.9 ($n = 5$; $\bar{x} \pm$ s.e. mean) percent of the maximum contraction produced by acetylcholine (10^{-5} M). Tetrodotoxin (1.6×10^{-6} M, $n = 6$) and hyoscine (2.6×10^{-6} M, $n = 6$) each reduced the effect of capsaicin (10^{-5} M) by 79% ($P < 0.001$, Student's t test). Tachyphylaxis to capsaicin was induced by the administration of a high concentration of the drug (1.6×10^{-4} M) for 10 min, 40 to 55 min before testing. This pretreatment caused a 90% reduction in the effect of a medium dose of capsaicin (10^{-5} M; $P < 0.001$, $n = 5$).

Periarterial stimulation (2 to 10 Hz for 30 s) invariably caused frequency-dependent contractions of preparations pretreated with guanethidine (1.7×10^{-5} M for 15 min, then 3.4×10^{-6} M throughout; Figure 1). Capsaicin tachyphylaxis markedly diminished the size and duration of these motor responses, while the residual responses were abolished by hexamethonium (1.8×10^{-4} M, $n = 6$; Figure 1). These findings, along with another six experiments, in which hexamethonium was given first, before the induction of capsaicin tachyphylaxis revealed an additive inhibitory effect of hexamethonium and capsaicin tachyphylaxis, which together abolished the response to periarterial stimulation (Figure 1). In the presence of hexamethonium, capsaicin-sensitive motor responses to periarterial stimulation at 10 Hz were inhibited by hyoscine (2.6×10^{-6} M) by $60.0 \pm 6.9\%$ ($P < 0.01$, $n = 5$).

The blocking effect of capsaicin tachyphylaxis was highly specific. While contractions to capsaicin or capsaicin-sensitive responses to periarterial stimulation showed no recovery for up to 3 h, contractions produced by nicotine ($2–6 \times 10^{-6}$ M, $n = 6$), cholinergic contractions (Gershon, 1967) to field stimulation (2 to 10 Hz for 10 s, $n = 4$), and the adrenergic relaxation produced by periarterial stimulation (2 to 20 Hz for 30 s, $n = 5$) remained completely unimpaired. The same was true for non-adrenergically mediated relaxation and rebound contraction (Gershon, 1967) evoked by field stimulation (5 to 20 Hz for 20 s), in the presence of hyoscine (2.6×10^{-6} M, $n = 4$).

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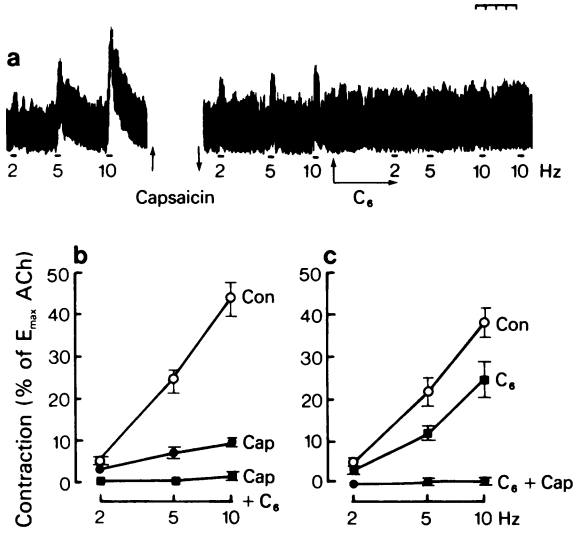

Figure 1 The effect of capsaicin tachyphylaxis and hexamethonium on the motor responses of the rabbit ileum to periarterial stimulation (2 to 10 Hz for 30 s, as indicated). (a) Capsaicin tachyphylaxis was induced by a 10 min application of the drug (1.6×10^{-4} M), followed by a 30 min washout period (↑ ↓). Hexamethonium (C_6) was added in a concentration of 1.8×10^{-4} M. Time scale: minutes. (b) and (c) Depression of the control responses to periarterial stimulation (2 to 10 Hz for 30 s) (Con) by capsaicin tachyphylaxis alone (Cap) and by the subsequent application of hexamethonium (Cap + C_6; $n = 6$), or by hexamethonium (C_6), followed by capsaicin tachyphylaxis, in the presence of hexamethonium (C_6 + Cap; $n = 6$). Data represent mean and s.e. mean; contractions are expressed as a percentage of the maximum contraction produced by acetylcholine (10^{-5} M). All segments were pretreated with guanethidine (1.7×10^{-5} M for 15 min; guanethidine 3.4×10^{-6} M was present throughout the experiment).

Discussion These findings indicate that contraction of the ileum of young rabbits to periarterial stimulation is mediated by two different neural mechanisms. The smaller, hexamethonium-sensitive component may be attributed to stimulation of preganglionic parasympathetic fibres. The major part of the motor response, however, was abolished by capsaicin tachyphylaxis. It was strongly inhibited by hyoscine, but not by hexamethonium.

Motor responses to capsaicin and periarterial stimulation have been described in the ileum (Barthó & Szolcsányi, 1978; Szolcsányi & Barthó, 1978) and taenia caeci of the adult guinea-pig. These responses were resistant to ganglionic blocking agents, but were abolished by capsaicin tachyphylaxis. Other neurally mediated intestinal responses, including relaxation to adrenergic periarterial stimulation and contractions to vagal preganglionic parasympathetic stimulation remained unimpaired by capsaicin tachyphylaxis (Szolcsányi & Barthó, 1978; 1979; 1980). It was concluded that capsaicin-sensitive contractions represented a new type of neurogenic, non-parasympathetic intestinal response, and the possible involvement of sensory neurones was proposed since capsaicin, a sensory neurone blocking agent, was capable of selectively stimulating and then blocking the function of one type of primary afferent neurones (see Szolcsányi & Barthó, 1978). The present findings on the rabbit ileum are consistent with this hypothesis; higher concentrations of capsaicin were needed in the rabbit than in the guinea-pig to produce either a contraction or tachyphylaxis.

In conclusion, these experiments suggest that a distinct, non-parasympathetic, capsaicin-sensitive motor mechanism is present in the rabbit small intestine.

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