Transmission from intramural inhibitory neurones to circular smooth muscle of the rabbit caecum and the effects of catecholamines

R. C. SMALL

Department of Pharmacology, University of Manchester

Preparations of circular smooth muscle from the rabbit caecum were mounted in a sucrose gap apparatus allowing the simultaneous recording of electrical and mechanical activity in response to field stimulation or drug administration.

The effects of stimulating intramural cholinergic neurones (Small, 1971) were abolished by superfusing the tissue with Krebs solution containing atropine 100 ng/ml or containing triethylcholine 2·5 mg/ml (preincubation for one hour during which the tissue was stimulated for 16 Hz for one second every 25 seconds). Tissues treated in either of these two ways responded to a single pulse of 0·5 ms duration with a wave of hyperpolarization. This response followed the stimulus artefact after a latent period of 151 ± 5 ms; the rise time of the wave of hyperpolarization was 307 ± 13 ms and its half decay time was 318 ± 20 ms (mean $\pm$ S.E., $n=13$ in each case). The time course of the response is therefore similar to that of inhibitory junctional potentials recorded from visceral smooth muscle by intracellular techniques (Bülbring & Tomita, 1967; Furness, 1969).

When trains of stimuli were applied (for one second every 100 sec) the individual waves of hyperpolarization began to summate at a stimulation frequency of 4 Hz and the peak hyperpolarization then increased with stimulation frequency up to a maximum at 64 Hz. Recovery from hyperpolarization was very rapid and often followed by a rebound wave of depolarization which, in the triethylcholine treated tissues, led to the firing of spikes and the development of tension.

The hyperpolarizing response of atropinized tissues was subjected to pharmacological analysis. It was abolished by tetrodotoxin 100 ng/ml. It could be observed in tissues from animals which had been treated with reserpine 2 mg/kg i.p. on each of two days prior to the experiment. It was resistant to guanethidine 2 μg/ml, phentolamine 2·8 μg/ml and propranolol 2·6 μg/ml. On the basis of this evidence it is suggested that the hyperpolarizing response results from the stimulation of intramural inhibitory neurones.

Drugs, in a dose volume of 0·05 ml, were injected into the flow of Krebs solution superfusing atropinized tissues. Noradrenaline (from $7{\cdot}4 \times 10^{-7}$ mole to $7{\cdot}4 \times 10^{-6}$ mole) caused a dose-dependent hyperpolarization and the maximum was less than that obtained by field stimulation. However, the effects of noradrenaline were slower to develop and to decay and could be antagonized by phentolamine.

Isoprenaline caused very little hyperpolarization, even when administered in doses as high as $0{\cdot}9 \times 10^{-6}$ mole.

These findings indicate that, in this tissue, the hyperpolarizing effects of catecholamines are mediated via α-adrenoceptors. A similar observation has been made for the guinea-pig taenia coli (Bülbring & Tomita, 1969). The hyperpolarization induced by field stimulation of the intrinsic inhibitory neurones is not mediated by the same receptor population as that induced by catecholamine administration.

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A preliminary investigation into the metabolic requirements of the contractile response of guinea-pig ileum to angiotensin

ANN D. CROCKER and K. A. WILSON* (introduced by M. D. DAY)

Department of Pharmacy, University of Aston in Birmingham, Birmingham B4 7ET

The contractile response of many smooth muscle preparations to angiotensin is well documented and has been reviewed recently (Khairallah, 1971). In this study the metabolic requirements for this action of angiotensin on guinea-pig ileum have been investigated.

The terminal ileum, removed from male albino guinea-pigs that had been starved overnight, was suspended in Tyrode solution containing 1 g/l. glucose at 28° C and gassed with air. Contractions were recorded on a kymograph using an isotonic, frontal-writing lever. In all experiments, concentrations of acetylcholine and angiotensin were selected which produced responses approximately equal to the 50% maximum acetylcholine response.

Initially the effect of altering the glucose concentration of the Tyrode solution, for 20 min periods, upon the responses to acetylcholine and angiotensin, was investigated. It was found that an increase to 8 g/l. glucose had no effect on the responses to either agonist. A decrease in glucose concentration to 0·25 g/l. reduced the response to acetylcholine by 12% but to angiotensin by 48%. The difference between the percentage reduction of the responses was highly significant ($P<0·001$). There was a significant difference ($P<0·001$) between the reduction of the acetylcholine response (60%) and the angiotensin response (92%) when glucose was completely removed from the Tyrode solution for 15 minutes. The effect of other hexoses was tested by replacing glucose in the Tyrode solution with either galactose or fructose at a concentration of 1 g/l. for 20 min. With galactose or fructose the responses to acetylcholine and angiotensin were similarly reduced (75–85%). These findings suggested that a glucose-dependent mechanism might be implicated in the angiotensin response.

The possibility that this mechanism utilized an oxidative pathway was investigated by comparing the responses to acetylcholine and angiotensin when the Tyrode was gassed with nitrogen for 110 min. There was a progressive reduction in the responses to both agonists which was greater for angiotensin (78%) than for acetylcholine (45%). Exposure of the tissue to carbon monoxide decreased the angiotensin response by 26% while the response to acetylcholine was unaffected or slightly increased.

These findings indicate that the contractile response of guinea-pig ileum to angiotensin might involve a glucose-dependent oxidative mechanism. Since it is well