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The action of sympathomimetic amines on circular and longitudinal smooth muscle from the isolated oesophagus of the guinea-pig

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The responses to sympathomimetic amines of circular and longitudinal preparations of smooth muscle from the isolated oesophagus of the guinea-pig have been investigated. Evidence is provided for the existence of α-excitatory and β-inhibitory adrenergic receptors in both circular and longitudinal smooth muscle preparations.

THERE are two macroscopically distinct muscle layers in the guinea-pig oesophagus, an outer striated and an inner layer of smooth muscle. Separation of the two layers is a simple process, enabling the pharmacology of the different types of muscle to be investigated independently. The present experiments concern the responses to sympathomimetic amines of longitudinal and circular strips from the smooth muscle of the guinea-pig oesophagus.

Experimental

Adult guinea-pigs were killed by stunning and bleeding. The oesophagus was removed and pinned on a cork mat under Krebs solution. The point of a pair of scissors was then inserted between the layers at the gastric end and the outer muscle coat was cut longitudinally and gently peeled away leaving an inner tube. This tube was opened by longitudinal incision and pinned flat with the mucosal surface uppermost. Longitudinal strips were cut parallel to the long axis to produce a length of 3 cm with a width of 3 mm.

Circular strips were prepared as described by Harry (1963) for the guinea-pig ileum; 5–6 cuts were necessary to produce a preparation 3 cm long, the width being 3 mm.

The preparations were set up in Krebs solution, aerated with oxygen 95% carbon dioxide 5% at 37°. Responses were recorded on a smoked drum using a light, side-writing lever with an initial load on the tissue of 300 mg for longitudinal strips and 200 mg for circular preparations. Magnification in all cases was 10.

Strips of either type were prepared from distal, medial and proximal regions of the oesophagus, no regional pharmacological variation being observed.

Some longitudinal preparations possessed inherent tone, which could be inhibited by drugs to give relaxation. Circular strips, however, always lacked tone and in order to demonstrate inhibitory responses it was necessary first to induce tone. Acetylcholine was suitable for this purpose, and was most effective when added to the bath to produce a final concentration of 1 μg/ml, 90 sec before the addition of the sympathomimetic amine, after which both drugs were washed out.

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DRUGS

Acetylcholine chloride (Hopkins and Williams). Adrenaline acid tartrate B.P. (Burroughs Wellcome). Dihydroergotamine methane sulphonate (Sandoz). Isoprenaline sulphate (Burroughs Wellcome). (–)-Noradrenaline bitartrate (Bayer). Pronethalol hydrochloride (I.C.I.). Concentrations are expressed as final bath concentration (μg/ml) in terms of the base.

The Krebs solution contained NaCl 0·692, KCl 0·0354, $CaCl_2$ 0·0282, $NaHCO_3$ 0·21, KH_2PO_4 0·0162, $MgSO_4.7H_2O$ 0·0294, glucose 0·2 g/100 ml.

Results

ADRENALINE, NORADRENALINE AND ISOPRENALINE ON THE LONGITUDINAL AND CIRCULAR MUSCLE STRIPS

Adrenaline in low doses (0·01–0·05 μg/ml) produced contraction of most longitudinal preparations. The response gradually changed to relaxation as the dose was increased until (at 4 μg/ml) relaxation was virtually maximal (Fig. 1). Noradrenaline produced a small contraction in some preparations at a concentration of 0·02 μg/ml. Relaxation responses appeared at a concentration of 0·04–0·1 μg/ml noradrenaline. Isoprenaline produced relaxation at 0·01 μg/ml (Fig. 1). Contraction was never observed with smaller doses.

In some preparations adrenaline failed to produce contraction. This phenomenon did not appear to be related to the initial tone of the preparation nor to the region of the oesophagus from which it was taken. Contraction to larger doses of adrenaline in the presence of pronethalol (see below) was typically obtained in these preparations.

All three sympathomimetic amines produced relaxation in circular muscle strips preparations with acetylcholine-induced tone. Approximately the same amount of inhibition was produced by 1 μg/ml noradrenaline, 2 μg/ml adrenaline or 0·1 μg/ml isoprenaline (Fig. 2).

EFFECTS OF ADRENERGIC BLOCKING DRUGS

Blockade of β-adrenergic actions: pronethalol. On longitudinal preparations pronethalol, at a concentration of 5 μg/ml, abolished isoprenaline relaxation and reversed the adrenaline or noradrenaline relaxation to contraction (Fig. 3). Similarly, on circular strips pronethalol antagonised the relaxation to the three sympathomimetic amines and in some experiments reversed the adrenaline response to a small contraction (Fig. 2).

Blockade of α-adrenergic actions. In longitudinal preparations piperoxan, at a concentration of 2 μg/ml, abolished the contractions to small doses of adrenaline (Fig. 1), or noradrenaline. Contractions to larger doses of adrenaline or noradrenaline, after reversal by pronethalol, were abolished by dihydroergotamine (10 μg/ml). Relaxation responses to adrenaline or noradrenaline and contractile responses to acetylcholine

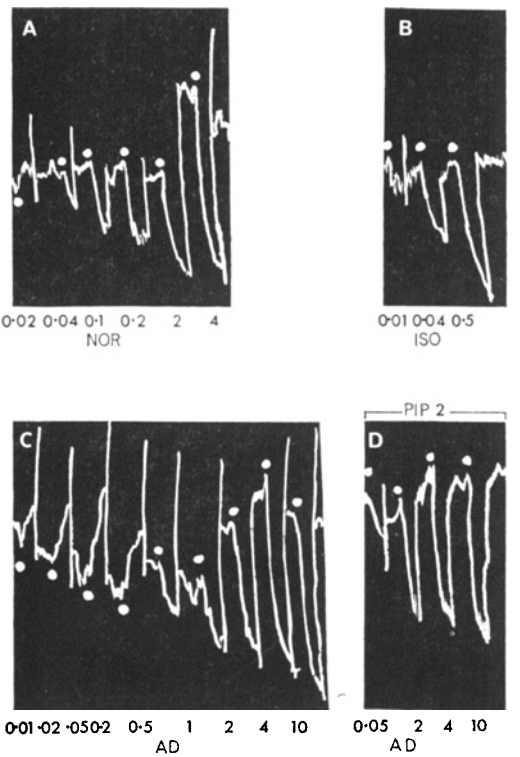

FIG. 1. Responses of the longitudinal smooth muscle preparation from the isolated oesophagus of the guinea-pig to noradrenaline (NOR), isoprenaline (ISO) and adrenaline (AD). The change from motor to inhibitory responses with increasing concentrations of noradrenaline in A or adrenaline in C is illustrated. Isoprenaline in B produced only inhibitory responses. Piperoxan (PIP 2 μg/ml) antagonised the motor but not the inhibitory responses to adrenaline in D. Concentrations are expressed as final bath concentrations (μg/ml) in terms of the drug base.

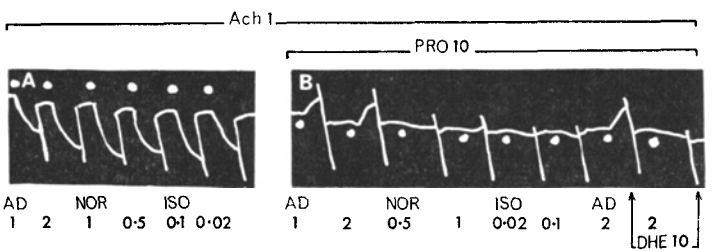

FIG. 2. The effect of sympathomimetic amines on the circular muscle preparation from the isolated oesophagus of the guinea-pig. Tone was induced with acetylcholine (Ach, 1 μg/ml). Adrenaline (AD), noradrenaline (NOR) or isoprenaline (ISO) produced inhibitory responses in A. In the presence of pronethalol (PRO 10 μg/ml in B) responses to noradrenaline or isoprenaline were abolished but adrenaline now produced a small motor response which was antagonised by dihydroergotamine (DHE). Concentrations are expressed as final bath concentrations (μg/ml) in terms of the drug base.

were not significantly modified by this concentration of dihydroergotamine (Fig. 4).

In circular muscle preparations dihydroergotamine antagonised the contraction produced by adrenaline in the presence of pronethalol (Fig. 2).

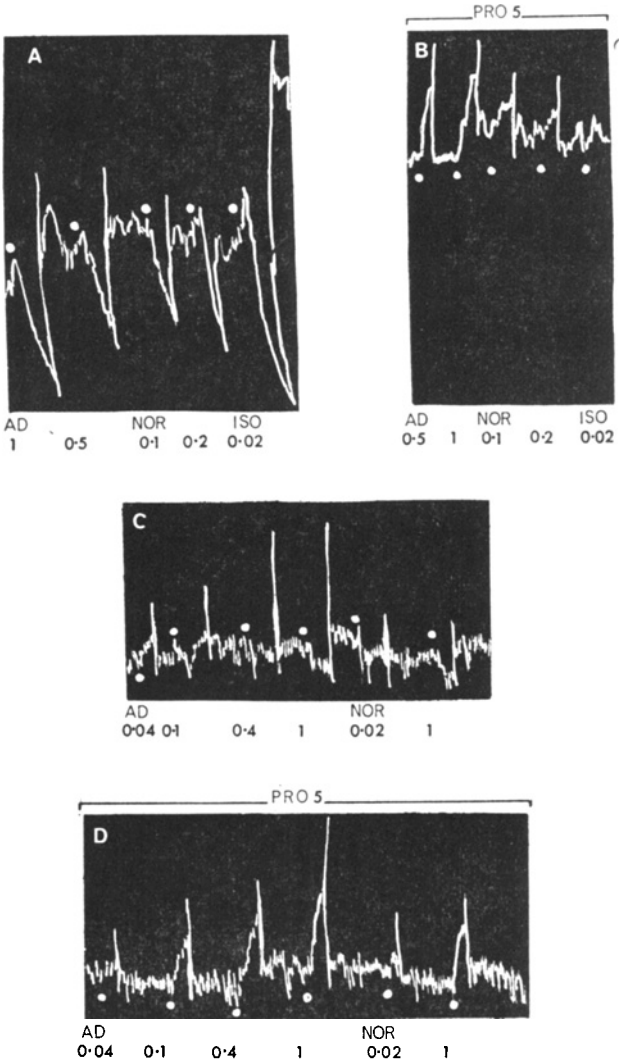

FIG. 3. The effect of pronethalol (PRO) on the responses of the longitudinal smooth muscle preparation from the isolated oesophagus of the guinea-pig, to sympathomimetic amines. In the preparation with inherent tone (see A) the inhibitory responses to adrenaline (AD) or noradrenaline (NOR) were reversed in the presence of pronethalol (5 μg/ml in B); the inhibitory response to isoprenaline was antagonised. In another preparation, with little tone (see C), adrenaline or noradrenaline produced little or no inhibitory effect; but after pronethalol in D, both drugs produced motor responses. Concentrations are expressed as final bath concentrations (μg/ml) in terms of the drug base.

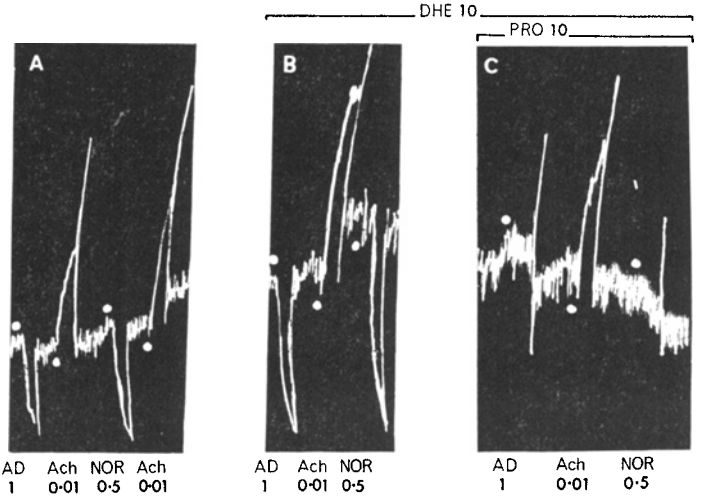

FIG. 4. The effect of dihydroergotamine (DHE) and pronethalol (PRO) on the responses of the longitudinal smooth muscle preparation from the isolated oesophagus of the guinea-pig to adrenaline (AD), noradrenaline (NOR) or acetylcholine (Ach). Responses in the absence of an antagonist are shown in A. In the presence of dihydroergotamine (10 μg/ml in B), the responses to the three drugs were not antagonised. Pronethalol (10 μg/ml) in the presence of dihydroergotamine in C abolished the inhibitory response to adrenaline or noradrenaline while the motor response to acetylcholine remained. Concentrations are expressed as final bath concentrations (μg/ml) in terms of the drug base.

Discussion

These experiments provide evidence for the presence of two kinds of adrenergic receptors in the smooth muscle of the oesophagus of the guinea-pig. These receptors correspond to the α- and β-actions defined by Ahlquist (1948, 1962). The receptor associated with the inhibitory response, whether of longitudinal or circular muscle strips, is most responsive to isoprenaline. The responses associated with this receptor are antagonised by pronethalol, a β-blocking agent (Black & Stephenson, 1962).

The receptor associated with contractile responses to sympathomimetic amines in both longitudinal and circular preparations, and which is unmasked by pronethalol, is most responsive to adrenaline. Furthermore, this receptor is blocked by the α-receptor antagonists dihydroergotamine or piperoxan. Thus there seem to be α-excitatory and β-inhibitory receptors in both circular and longitudinal components of the smooth muscle of the guinea-pig oesophagus.

Turning to the effect of concentration of the sympathomimetic amines, it was noticeable that on the longitudinal muscle preparation the α-effects were seen with lower concentrations, yet the β-effects, although initiated only at a higher threshold, predominated. A change from excitation to inhibition with increasing doses of adrenaline is an effect apparently not hitherto reported for the gastrointestinal tract. Various workers have

reported adrenaline-induced motor responses of muscularis mucosa from differing areas of the gut. Thus, Burnstock (1960) demonstrated adrenaline contractions of the muscularis mucosa of the domestic pig oesophagus. Walder (1953) found that adrenaline produced a contraction of some regions of the muscularis mucosa of human stomach which was antagonised by ergotamine. Similarly with preparations of the muscularis mucosa of the small intestine of the dog, King & Robinson (1945), and also King, Glass & Townsend (1947), observed contraction of the circular and longitudinal preparations, induced by adrenaline which could be antagonised by ergotamine but not by atropine. Adrenaline motor effects have also been reported in isolated segments of the terminal ileum of the guinea-pig (Munro, 1951).

In the gastrointestinal tract, adrenergic receptors, whether of α- or β-type reaction, are generally associated with inhibitory responses. However, in many other tissues the occurrence of α-excitatory and β-inhibitory receptors has been also demonstrated. Vascular smooth muscle is an obvious example; others include the isolated nictitating membrane of the cat (Thompson, 1958), the uterus of the guinea-pig (Hermansen, 1960), the bronchioles of the anaesthetised dog (Castro de la Mata, Penna & Aviado, 1962), the isolated junction of the bile duct and the duodenum (Crema & Berté, 1963) and the isolated vas deferens hypogastric nerve preparation (Holman & Jowett, 1964; Large, 1965).

It may be noted that the relative predominance of α- or β-effects varies from preparation to preparation. α-Excitation is predominant in the vas deferens, the nictitating membrane and the bile duct. β-Inhibition predominates in the bronchioles and also, as reported here, in the oesophagus.

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