J. Pharm. Pharmacol., 1964, **16**, 332–336 Received December 10, 1963

The site of action of sympathomimetic amines on the circular muscle strip from the guinea-pig isolated ileum

J. HARRY

The circular muscle strip of the isolated ileum of the guinea-pig, treated with the anticholinesterase mipafox (100 μg/ml) responds to doses of methacholine or carbachol with contractions that are inhibited by previous doses of noradrenaline, adrenaline or isoprenaline in that order of potency. Piperoxane antagonised the inhibitory action of adrenaline, but not that of aminophylline. Dichloroisoprenaline did not antagonise the action of adrenaline on the circular muscle strip. It is concluded that the site of action of the sympathomimetic amines on the circular muscle of the guinea-pig ileum is located at postganglionic neuro-effector junctions in the smooth muscle.

THE study of the actions of sympathomimetic amines on the circular muscle of the guinea-pig isolated ileum has been restricted to the effect of these amines on peristalsis (Pirie, 1951; McDougal & West, 1952, 1954; Kosterlitz & Robinson, 1957). Harry (1963) suggested that peristalsis does not lend itself to an accurate evaluation of drug action and introduced the circular muscle strip for this purpose. The site of action of sympathomimetic amines on the circular muscle strip is now reported.

Methods

Segments 1·5 cm in length were taken from the ileum of a guinea-pig 15 cm from the ileo-caecal junction. A segment was opened by a longitudinal incision through the wall along its mesenteric border and the resultant rectangle of ileum was pinned out under Krebs solution with the mucosal surface upwards. A strip was produced by cutting the rectangle in the direction of the circular muscle fibres (Harry, 1963). Usually five cuts produced a strip of circular muscle of sufficient length. A cotton ligature was tied around each end of the strip, one of which was tied to a glass holder and the other to a small metal hook. The holder was immersed in an organ bath containing 23 ml of Krebs solution at 37° and bubbled with 95% oxygen and 5% carbon dioxide. The metal hook was attached to an isotonic lever with a frontal writing point. The load on the tissue was 290 mg; the response of the tissue was magnified six times.

The circular muscle strip lacks a tone which sympathomimetic amines could relax, thus making it impossible to demonstrate directly the inhibitory actions of these amines. A suitable method was to obtain a series of contractions, 90% of the maximum, of the circular strip by methacholine or carbacol and to inhibit these contractions with the sympathomimetic agent. A satisfactory cycle was as follows: (1) the spasmogen was added to the bath for a contact time of 1 min; (2) the bath fluid was changed; (3) after a further min the bath fluid was changed again; (4) 1 min after the second washing the spasmogen was added to the bath

From the Department of Pharmacology, King's College, Strand, London, W.C.2.

again and the cycle repeated until a series of contractions of constant height was achieved. The effect of the sympathomimetic agent was tested by adding it to the bath immediately after the second washing at (3). In this way the sympathomimetic drug was present in the bath fluid for 1 min before the spasmogen was added. When the activity of a potential sympathomimetic antagonist was investigated, this substance was added to the bath fluid each time after it was changed.

DRUGS USED

Adrenaline acid tartrate, aminophylline, carbachol chloride, dichloroisoprenaline hydrochloride, isoprenaline sulphate, methacholine chloride, piperoxane hydrochloride, phenoxybenzamine hydrochloride.

All drugs were prepared in Krebs solution from stock solutions in distilled water and are expressed as base.

Results

THE EFFECTS OF ADRENALINE, NORADRENALINE AND ISOPRENALINE ON THE CIRCULAR MUSCLE STRIP

Adrenaline, noradrenaline and isoprenaline all produced inhibition of the contractions of the circular muscle strip caused by 1·0 μg/ml carbachol (Fig. 1). The same degree of inhibition was produced by 10 ng/ml of noradrenaline, by 20 ng/ml of adrenaline and by 4000 ng/ml of isoprenaline.

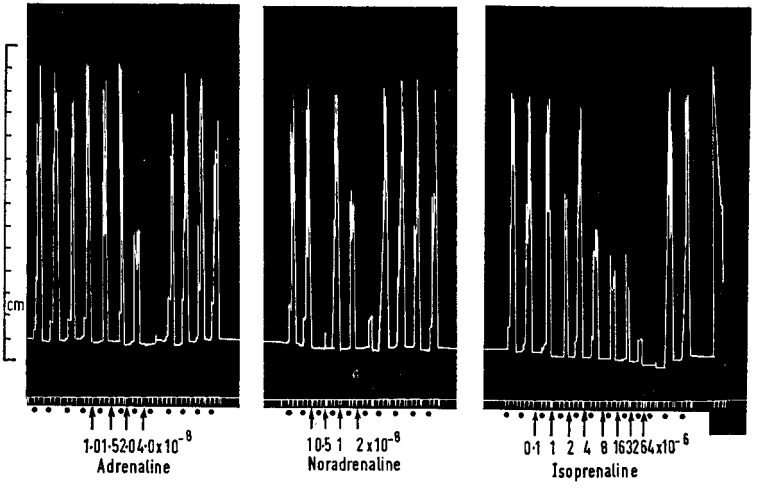

FIG. 1. The inhibitory effects of adrenaline, noradrenaline and isoprenaline on the contraction of the circular muscle strip produced by carbachol. At ● carbachol added to the bath fluid to give a concentration of 1·0 μg/ml. Sympathomimetic drugs added to bath fluid at arrows. Bath concentrations expressed as g/ml. Time marker, 30 sec.

THE EFFECT OF ADRENALINE ON THE CIRCULAR MUSCLE STRIP

Adrenaline, 0·1 μg/ml, inhibited the contractions of the circular muscle strip produced by 1·0 μg/ml of methacholine (Fig. 2). This inhibitory action of adrenaline was antagonised by piperoxane, 2·0 μg/ml, a concentration which did not affect the contraction of the strip by methacholine (central portion of Fig. 2). The inhibitory action of adrenaline returned after washing the piperoxane from the tissue (right portion of Fig. 2). Phenoxybenzamine, 0·1 μg/ml, also antagonised the action of adrenaline on the circular muscle strip.

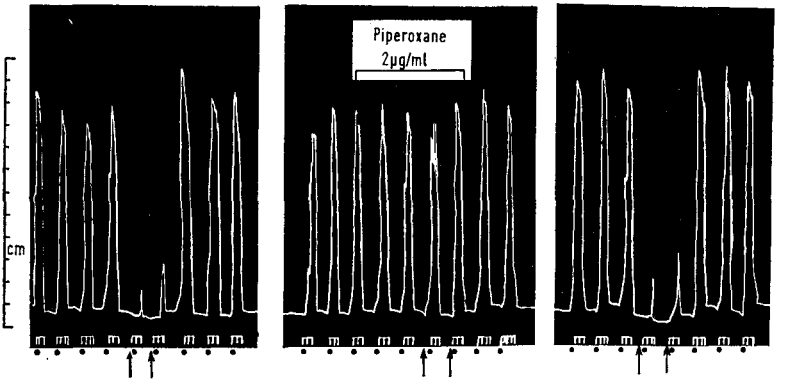

FIG. 2. The effect of piperoxane on the inhibitory action of adrenaline. At ● methacholine added to the bath fluid to give a bath concentration of 1·0 μg/ml. At arrows, adrenaline added to the bath fluid to give a bath concentration of 0·1 μg/ml. ⌐ shows presence of piperoxane 2·0 μg/ml in the bath fluid. Time marker, 30 sec.

THE EFFECT OF AMINOPHYLLINE ON THE CIRCULAR MUSCLE STRIP

Aminophylline, 67·5 μg/ml, inhibited the contractions of the circular muscle strip produced by methacholine, and this action of aminophylline was not influenced by piperoxane, 2·0 μg/ml, a concentration which inhibited the action of adrenaline (Fig. 3).

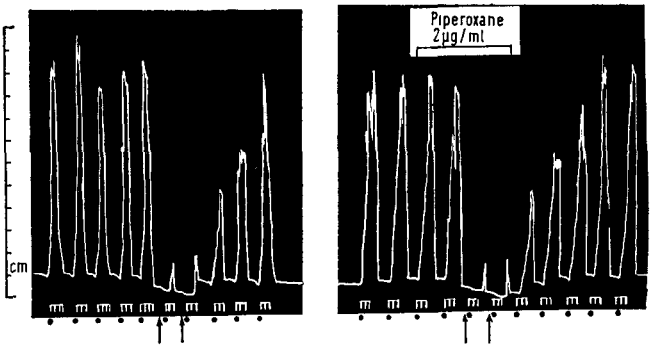

FIG. 3. The effect of piperoxane on the spasmolytic action of aminophylline. At ● methacholine added to the bath fluid to give a bath concentration of 1·0 μg/ml. At arrows, aminophylline added to bath fluid to give a bath concentration of 67·5 μg/ml. ⌐ shows presence of piperoxane (2·0 μg/ml) fluid. Time marker, 30 sec.

THE EFFECT OF DICHLOROISOPRENALINE ON THE SPASMOLYTIC ACTIVITY OF ADRENALINE

Dichloroisoprenaline (DCI), 1·0 μg/ml, did not have any spasmolytic activity on contractions of the circular muscle strip produced by methacholine, 2·0 μg/ml, and did not modify the inhibitory action of adrenaline (Fig. 4).

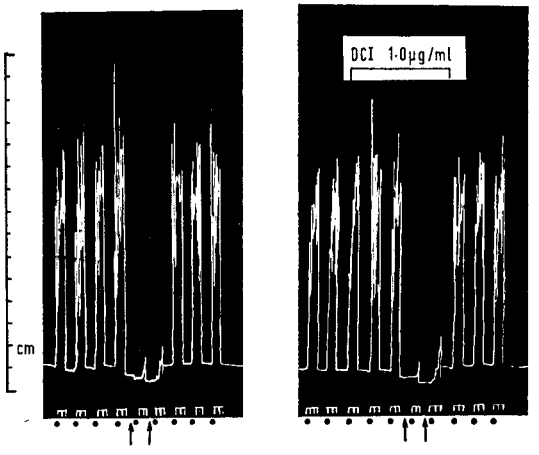

FIG. 4. The effect of dichloroisoprenaline (DCI) on the inhibitory action of adrenaline. At ● methacholine added to bath fluid to give a concentration of 2·0 μg/ml. At arrow, adrenaline added to bath fluid to give a concentration of 0·1 μg/ml ⌐ shows presence of DCI (1·0 μg/ml) in bath fluid. Time marker, 30 sec.

Discussion

The results of these experiments show that the circular muscle of the guinea-pig isolated ileum possesses adrenotropic receptors which are inhibitory. Further the receptors can be classified as α-receptors (Alquist, 1948). Three pieces of evidence are offered in support. Firstly, noradrenaline was more active than adrenaline and both of these substances were much more active than isoprenaline on the circular muscle strip; secondly the inhibitory action of adrenaline but not aminophylline was specifically antagonised by the α-receptor blocking agent piperoxane, and thirdly dichloroisoprenaline, which antagonises the β-receptor actions of adrenaline (Powell & Slater, 1958; Alquist & Levy, 1959), did not modify the inhibitory action of adrenaline on the circular muscle strip.

The observation that hexamethonium did not influence periarterial sympathetic stimulation of a segment of isolated guinea-pig ileum (Szerb, 1961) suggested that the efferent sympathetic chain is not functionally related to the enteric plexuses of the ileum but terminates in the intestinal musculature. Taken together with the results reported above this allows me to conclude that the site of action of noradrenaline, adrenaline and isoprenaline on the circular muscle of the guinea-pig ileum is located at post-ganglionic neuro-effector junctions in the smooth muscle. These

conclusions differ from those of McDougal & West (1954). But their interpretations of the action of sympathomimetic amines on peristalsis from observations on the longitudinal tubular preparation of the guinea-pig isolated ileum, led them to infer that the intramural nerve plexus was involved. However, Brownlee & Harry (1963) have shown a basic difference pharmacologically between the longitudinal and circular muscle layers of the guinea-pig ileum and thus the inferences of McDougal & West concerning the site of action of sympathomimetic amines on circular muscle activity from experiments on the longitudinal muscle must now be reconsidered.

Acknowledgement. I am greatly indebted to Professor G. Brownlee for his help and advice throughout this work and in the preparation of this manuscript.

References

Ahlquist, R. P. A. (1948). *Amer. J. Physiol.*, **153**, 586–600.
Ahlquist, R. P. A. & Levy, B. (1959). *J. Pharmacol.*, **127**, 146–149.
Brownlee, G. & Harry, J. (1963). *Brit. J. Pharmacol.*, **21**, 544–554.
Harry, J. (1963). *Ibid.*, **20**, 399–417.
Kosterlitz, H. W. & Robinson, J. A. (1957). *J. Physiol.*, **136**, 249–262.
McDougal, M. D. & West, G. B. (1952). *Arch. int. Pharmacodyn.*, **90**, 86–92.
McDougal, M. D. & West, G. B. (1954). *Brit. J. Pharmacol.*, **9**, 131–137.
Pirie, V. (1951). *J. Physiol.*, **115**, 63P.
Powell, C. E. & Slater, I. H. (1958). *J. Pharmacol.*, **122**, 480–488.
Szerb, J. C. (1961). *Brit. J. Pharmacol.*, **16**, 23–31.