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Research Papers

A preliminary investigation of the pharmacology of longitudinal muscle strips from human isolated jejunum

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The effects of drugs on longitudinal muscle strips of human jejunum were studied *in vitro*. The muscarinic site of action of acetylcholine was demonstrated. The sympathomimetic amines phenylephrine, noradrenaline and isoprenaline each produced only a relaxation by an action on adrenergic α- and β-receptors. The presence of both types of receptor was demonstrated by selective adrenergic blockade with pronethalol or Hydergine. The ganglion stimulating agent dimethylphenylpiperazinium produced a contraction by an action on intramural cholinergic nervous tissue. When the contractile response was blocked by hyoscine, a relaxation occurred, due to the stimulation of an adrenergic mechanism which could be either the sympathetic nerves of the intrinsic nerve plexus or adrenergic stores in the bowel wall. The contraction produced by histamine was not inhibited by hyoscine or hexamethonium but was blocked by mepyramine, thus indicating a direct effect of the drug on the smooth muscle. 5-Hydroxytryptamine produced a contraction by a direct action on the muscle, since the response was not inhibited by hyoscine or hexamethonium but was blocked by methysergide (UML 491).

THE effects of drugs on human oesophageal muscle have been studied by Ellis, Kauntze, Nightingale & Trounce (1960), and Trounce & Nightingale (1960) made similar experiments on normal and diseased colonic muscle as part of an investigation of Hirschsprung's disease. The pharmacology of the circular muscle of the human colon has been recently reported by Fishlock & Parks (1963) and the human isolated taenia coli preparation has also been examined by Bucknell & Whitney (1964). The present paper reports the results of a preliminary investigation of the pharmacology of longitudinal strips of human isolated jejunum.

Experimental

Methods

Longitudinal strips were cut from the jejunum at operations in which gastro-jejunostomy was being performed for duodenal ulcer or carcinoma of the stomach. The specimen was immediately placed in cooled Krebs solution containing half the usual concentration of calcium, and brought to the laboratory. The composition of the modified Krebs solution expressed in g/litre was NaCl 6·9 ,KCl 0·35, $CaCl_2.6H_2O$ 0·27, KH_2PO_4 0·16, $MgSO_4.7H_2O$ 0·29, (+)-glucose 1·0 and $NaHCO_3$ 2·1.

In most experiments the mucosa was removed and the longitudinal muscle strip set up in a 15 ml organ-bath containing the modified Krebs solution at 37°, and gassed with 5% carbon dioxide in oxygen. In a few experiments the strip was set up without first removing the mucosa; no differences in response were observed between tissues with and without

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mucosa. The muscle strip was usually about 30 mm long by 1 mm wide. Recordings were made on a smoked drum using a frontal writing isotonic lever. The load on the tissue was 1 g. The responses were magnified eight times. Tissue was often stored overnight in modified Krebs solution at 4°, when only minimal changes in sensitivity were seen, or left set up in the organ bath at 37°. Often the "tone" was not high enough to show a relaxation, and in these experiments the inhibitory effect of the sympathomimetic amines was demonstrated as a reduction in standard responses to acetylcholine or 5-HT.

Drugs. Acetylcholine perchlorate, (−) noradrenaline bitartrate, (±) isoprenaline sulphate, (±)-phenylephrine hydrochloride, 5-hydroxytryptamine creatinine sulphate (5-HT), histamine acid phosphate, dimethylphenylpiperazinium iodide (DMPP), physostigmine sulphate, (−)-hyoscine hydrobromide, mepyramine maleate, procaine hydrochloride, hexamethonium bromide, pronethalol hydrochloride, Hydergine (a mixture of equal parts of dihydroergocornine, dihydroergocryptine, and dihydroergocristine as methanesulphonate) and methysergide hydrogen maleate (UML 491). Drug concentrations are expressed as μg/ml of the final bath concentration of the base, with the exception of DMPP, phenylephrine, procaine, pronethalol, Hydergine and methysergide, which are expressed as the salt.

Results

Spontaneous activity

Most preparations exhibited large, regular spontaneous activity. The lowest point of the wave often corresponded with maximal relaxation of the tissue. Krebs solution containing half the usual amount of calcium reduced the amplitude of the spontaneous activity, but not the responses of the tissue to the various drugs.

Actions of acetylcholine

The tissue contracted to acetylcholine in a concentration range of 0·01–0·2 μg/ml. Physostigmine, 0·3 μg/ml, left in contact with the tissue for 10 min, potentiated the effect of acetylcholine. The contractile response to acetylcholine was not modified by hexamethonium, 30 μg/ml, which completely blocked a response to DMPP at 10 μg/ml. Exposure of the tissue to hyoscine, 0·01 μg/ml, for 3 min completely inhibited the effect of the largest dose of acetylcholine in the range of 0·01–0·2 μg/ml used to establish the dose response curve.

Actions of phenylephrine, noradrenaline and isoprenaline

The sympathomimetic amines phenylephrine, noradrenaline and isoprenaline relaxed the tissue and inhibited spontaneous activity. The range of potency of the sympathomimetic amines sometimes changed when the tissue was kept overnight at 37°. In one experiment noradrenaline 0·02 μg/ml, was equivalent to isoprenaline 0·2 μg/ml when the

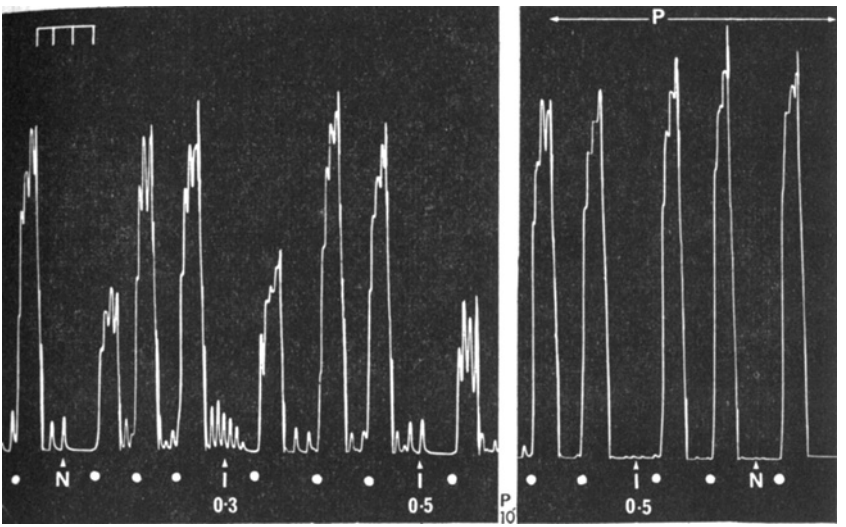

FIG. 1. The effect of pronethalol (P) on the response of the longitudinal strip of jejunum to noradrenaline (N,0·03 μg) or isoprenaline (I, μg). l.h. panel shows the reduction in the standard response to acetylcholine (0·015 μg at white dots) when noradrenaline or isoprenaline was added to the bath 30 sec before the dose of agonist. After incubation of the tissue with pronethalol (2 μg/ml for 10 min), r.h. panel the inhibitory effect of noradrenaline or isoprenaline, on the response to acetylcholine (0·045 μg), no longer occurred. Time signal 30 sec.

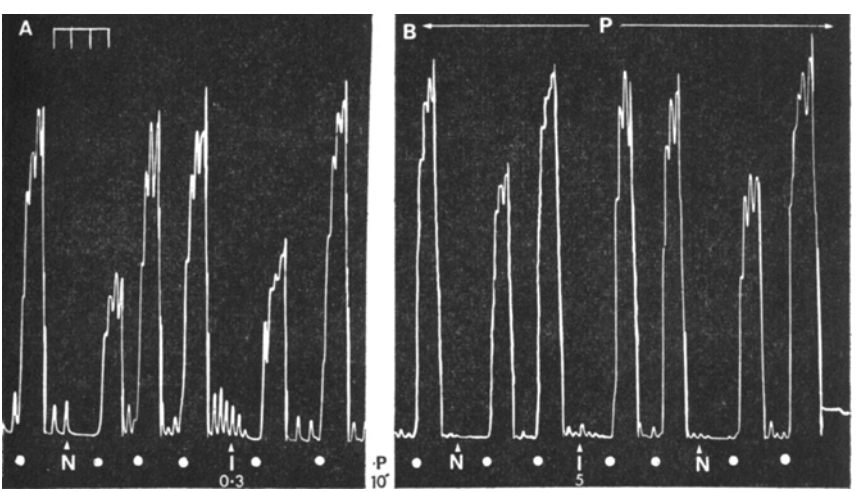

FIG. 2. The effect of pronethalol (P, 0·2 μg) on the responses of the longitudinal strip of human jejunum to noradrenaline (N, 0·03 μg) or isoprenaline (I, μg). Panel A shows the reduction in the standard response to acetylcholine (0·02 μg at white dots) when noradrenaline or isoprenaline was added to the bath 30 sec before the addition of the dose of agonist. Panel B shows the same procedure after incubation of the tissue with pronethalol, 0·2 μg/ml for 10 min. The inhibitory effect of noradrenaline was reduced and that of isoprenaline was abolished. Time signal 30 sec.

tissue was fresh, but the following day the sensitivity of the same piece of tissue was such that isoprenaline 0·7 μg/ml was equivalent to noradrenaline 3 μg/ml. After an initial exposure of the tissue to pronethalol, 2 μg/ml, for 10 min, and subsequently for 3 min before each addition of acetylcholine, the inhibitory effect of noradrenaline and isoprenaline on the contractile response to acetylcholine was abolished (Fig. 1). When 10% of the concentration of pronethalol was used (0·2 μg/ml) the inhibitory effect of isoprenaline was abolished whereas the effect of noradrenaline was only slightly reduced (Fig. 2). Initial exposure of the tissue to Hydergine, 5 μg/ml, for 5 min, and subsequently for 3 min before each addition of acetylcholine, abolished the effect of phenylephrine on the contractile response to acetylcholine, whereas the inhibitory effect of isoprenaline was only slightly reduced.

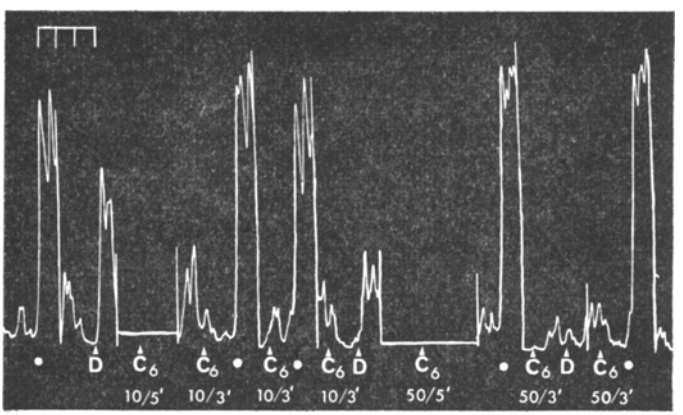

FIG. 3. The effect of hexamethonium (C6, μg/time) on responses of the longitudinal strip of jejunum to DMPP (D, 15 μg). The contractile response to DMPP was reduced by hexamethonium 10 μg/ml and abolished by 50 μg/ml leaving the response to acetylcholine unchanged. Time signal 30 sec.

ACTIONS OF DMPP

DMPP, 10 μg/ml, caused a contraction of the tissue. Minimal responses were obtained with 2 μg/ml and maximal responses with 40 μg/ml. Incubation of the tissue with eserine 0·5 μg/ml, for 5 min, potentiated the response to DMPP by a factor of five. Hexamethonium, 10 μg/ml, reduced the response, and 50 μg/ml caused complete inhibition (Fig. 3). The response to DMPP returned 30 min after hexamethonium had been removed from the bath. Hyoscine, 0·01 μg/ml, blocked the response to DMPP and in some experiments the response to DMPP was converted from a contraction to a relaxation in the presence of hyoscine (Fig. 4A, B). The contractile response was not changed by the removal of the mucosa.

The relaxant response to DMPP, 30 μg/ml, obtained in the presence of hyoscine, 0·05 μg/ml, was abolished after exposure of the tissue to hexamethonium, 50 μg/ml, for 5 min.

ACTIONS OF 5-HT

The tissue responded with a contraction to 5-HT in a concentration range of 0·05 μg–1 μg/ml. The response was not affected by hyoscine, 0·01–1·0 μg/ml (Fig. 4C), mepyramine, 0·01 μg/ml, which inhibited the response to histamine, or by hexamethonium, 50 μg/ml, which blocked the contractile response to DMPP (Fig. 5A, B). Methysergide, 1 μg/ml, completely inhibited the response to 5-HT, leaving the response to acetylcholine unchanged (Fig. 5C).

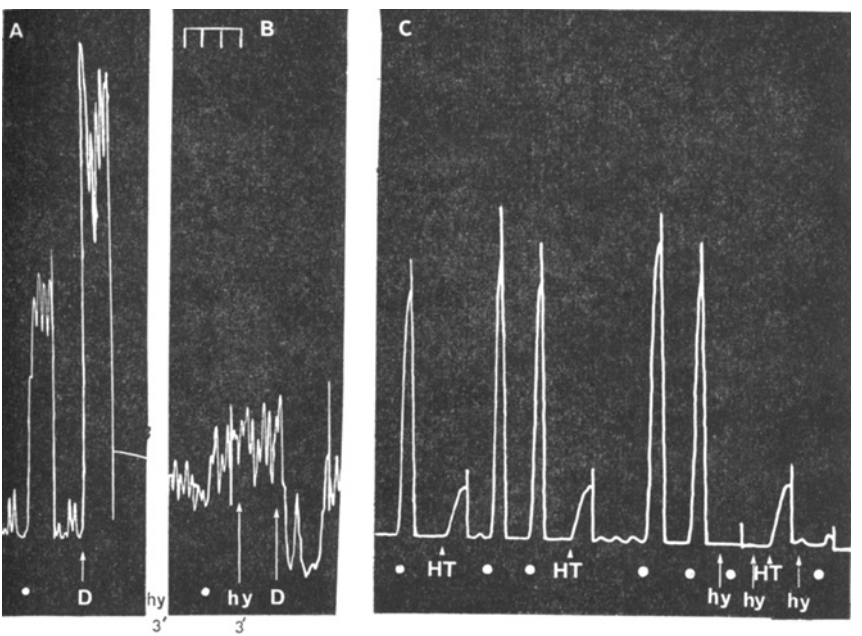

FIG. 4. The effect of hyoscine on the responses of the longitudinal strip of jejunum to DMPP and to 5-HT. Panel A shows the contractile response to acetylcholine (0·03 μg at white dot) and DMPP (D, 15 μg). After incubation with hyoscine (hy, 0·01 μg/ml) for 3 min, panel B, the response to acetylcholine was abolished and the response to DMPP was converted to a relaxation. Panel C shows the contractile response to acetylcholine (0·5 μg at white dots) was inhibited by hyoscine (hy, 0·01 μg/ml for 3 min) whereas the response to 5-HT (2 μg) was unchanged. Time signal 30 sec.

ACTIONS OF HISTAMINE

The tissue responded to histamine with a contraction in a concentration range of 0·5–40 μg/ml. Neither hexamethonium, 33 μg/ml (Fig. 6), nor hyoscine, 0·05 μg/ml, modified the response to histamine. Mepyramine, 0·01 μg/ml for 5 min, completely inhibited the response to histamine, but did not affect the response to acetylcholine or 5-HT (Fig. 7).

Discussion

The preparations of jejunum used were obtained from surgical intervention in man. In no instance was the jejunum itself involved in disease,

and since reproducible responses to drug doses were obtained, the results are considered to represent the pharmacology of longitudinal strips of muscle of normal human isolated jejunum.

The preparation was usually freed from mucosa to eliminate the possibility of a mechanical barrier to the diffusion of drugs; also the isolated mucosa of the human stomach secretes a substance which inhibits the action of drugs (Walder, 1953). Whether the removal of the mucosa and submucosa so damaged most of the intramural nervous plexus that the responses to drugs acting on receptors sited in the nervous tissue were lost, was challenged in experiments in which the jejunum was prepared with the mucosa intact. In these experiments no differences were seen in the responses to either acetylcholine or DMPP when the mucosa was subsequently removed.

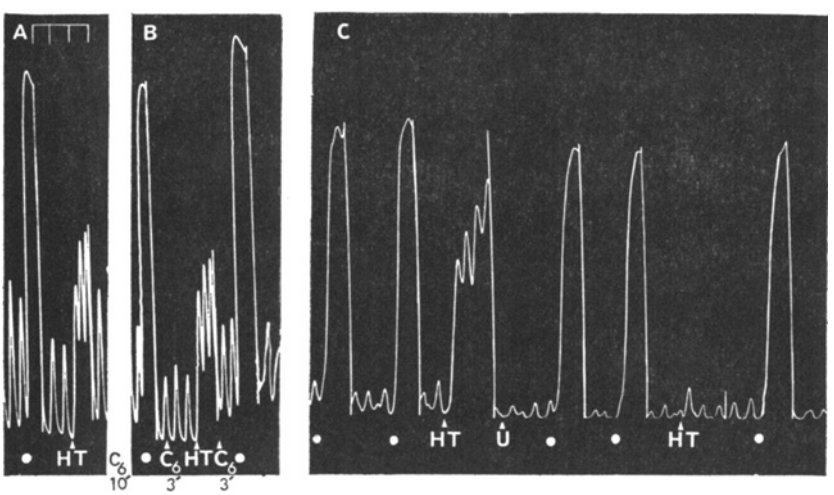

FIG. 5. The effect of hexamethonium and methysergide on the responses of the longitudinal strip of jejunum to 5-HT. Panels A and B show the contractile responses to acetylcholine (0·4 μg at white dots) and 5-HT (1 μg) were not modified by incubation of the tissue with hexamethonium (C6, 50 μg) for 10 min and for 3 min before the test drug. Panel C shows that incubation of the tissue with methysergide (U, 1 μg/ml) for 18 min did not modify the response to acetylcholine (0·1 μg at white dots) whereas the contractile response to 5-HT (0·5 μg) was abolished. Time signal 30 sec.

Acetylcholine produced typical muscarinic effects on human jejunum, the contractile response being blocked by hyoscine, potentiated by physostigmine and unaffected by hexamethonium.

Phenylephrine, noradrenaline or isoprenaline showed only inhibitory effects on the tissue, demonstrated as a reduction in the contractile response to acetylcholine. The inhibitory effect of isoprenaline was abolished by the β-receptor blocking agent pronethalol (Black & Stephenson, 1962) in a concentration which did not affect the response to noradrenaline. Conversely, the α-receptor blocking agent Hydergine blocked the effect of phenylephrine, whereas the response to isoprenaline was only

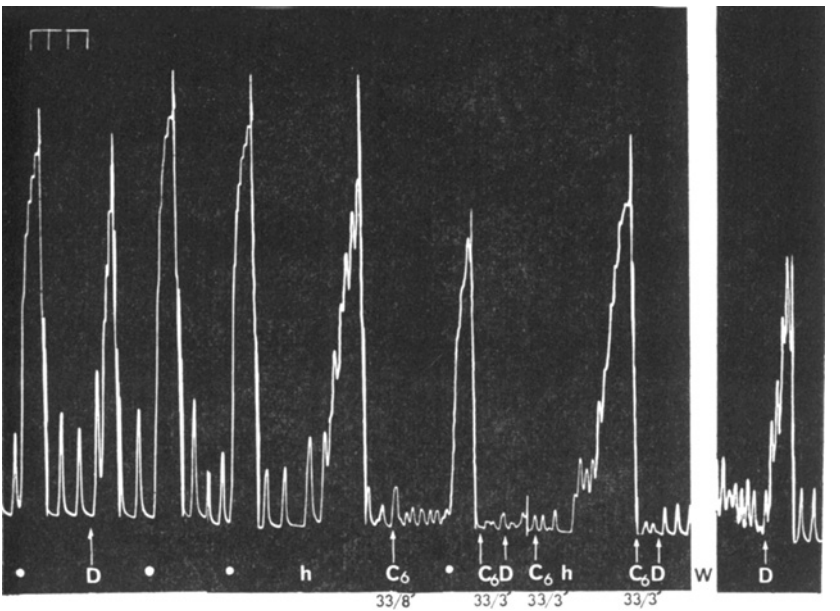

FIG. 6. The effect of hexamethonium on the responses of the longitudinal strip of jejunum to histamine or DMPP. Contractile responses to acetylcholine (0·2 μg at white dots), DMPP (D, 5 μg) and histamine (h, 0·07 μg). After incubation with hexamethonium (C6 μg/time), there was no response to DMPP whereas the responses to acetylcholine or histamine were unchanged. The response to DMPP returned 30 min after hexamethonium had been removed from the bath. Time signal 30 sec.

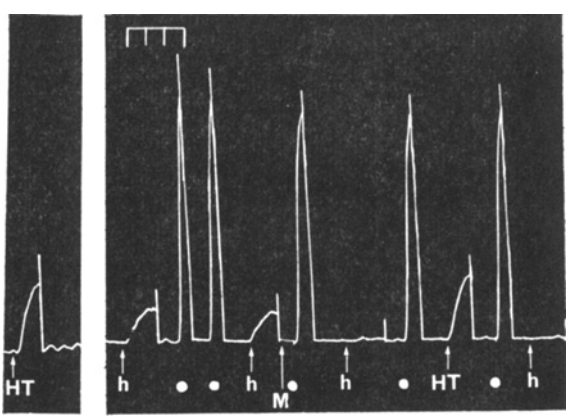

FIG. 7. The effect of mepyramine on the responses of the longitudinal strip of jejunum to 5-HT or histamine. Contractile responses to 5-HT (2 μg) histamine (h, 4 μg) and acetylcholine (0·5 μg at white dots) are shown on the left of the tracing. After incubation of the tissue with mepyramine (M, 0·01 μg/ml) for 5 min, there was no response to histamine whereas the responses to acetylcholine or 5-HT were not reduced. Time signal 30 sec.

reduced. Since phenylephrine acts mainly on the α-receptors, noradrenaline on α- and β-receptors, and isoprenaline on the β-receptors (Ahlquist & Levy, 1959; Levy & Ahlquist, 1961; Kosterlitz & Lees, 1964), the selective blockade of the responses to these drugs by pronethalol or Hydergine suggests the presence of both α- and β-adrenergic receptors in the human jejunum and that stimulation of either produces a relaxation. The concept of two types of receptor was first put forward by Ahlquist (1948) using the range of potency of six closely related sympathomimetic amines on various tissues, and his experiments showed a fixed range of potency for each particular tissue. However, in human jejunum the range of potency for noradrenaline and isoprenaline was not constant, and in one experiment the relative potency reversed when the tissue was stored overnight. This reversal involved a 100-fold decrease in sensitivity to noradrenaline as compared with a 3-fold decrease in sensitivity to isoprenaline.

The ganglion stimulating drug, DMPP, produced a contraction of the tissue which was blocked at neuronal sites by hexamethonium and peripherally by hyoscine. When the contraction was blocked by hyoscine, the response to DMPP was converted to a relaxation. The change in response from a contraction to a relaxation was not obtained by varying the concentration of DMPP as was seen when nicotine was used on the rabbit colon (Gillespie & Mackenna, 1960). The relaxation produced by DMPP in the presence of hyoscine was blocked by hexamethonium, suggesting a neuronal site of action. The ability of the tissue to respond to DMPP with either a contraction or a relaxation demonstrated the presence of both cholinergic and adrenergic nervous tissue in the wall of the human jejunum, and that the cholinergic component was dominant since the adrenergic component was only revealed in the presence of hyoscine. These results are similar to those described for rabbit and kitten ileum by Ambache & Edwards (1951), but differ from those of human colon where only an adrenergic response was demonstrated (Bucknell & Whitney, 1964). The relaxant action of DMPP does not necessarily imply the presence of ganglia with postganglionic adrenergic neurones, since the possibility that the relaxation resulted from an action on adrenergic nerve terminals has not been experimentally excluded.

Histamine has been shown to have a direct action on guinea-pig ileum (Day & Vane, 1963), and an action on both muscle and intramural nervous tissue in the rabbit (Ambach & Lessin, 1955). Brownlee & Harry (1963) have shown a difference between longitudinal and circular muscle strips of the guinea-pig ileum, histamine having a direct action on the longitudinal strips and an indirect action on the circular ones. Longitudinal strips of human jejunum responded to histamine with a contraction due to a direct action, since it was not affected by hexamethonium or by hyoscine. Further support for a direct action of histamine was obtained by selective blockade of the contractile response to histamine by mepyramine.

5-HT has been shown to act on receptors sited both on nervous tissue and on muscle in guinea-pig ileum (Gaddum & Picarelli, 1957). Brownlee

& Johnson (1963) and Day & Vane (1963) showed that 5-HT contracted the longitudinal muscle of guinea-pig ileum mainly by an action through a cholinergic nerve pathway. However, the present experiments on longitudinal strips of human jejunum showed only a direct action of 5-HT, since the contractile response was not inhibited by hexamethonium or by hyoscine. The direct action was confirmed by blockade of the contractile response by methysergide, a substance which is a potent antagonist of the direct actions of 5-HT on smooth muscle.

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References

Ahlquist, R. P. (1948). *Amer. J. Physiol.*, **153**, 586–600.
Ahlquist, R. P. & Levy, B. (1959). *J. Pharmacol. exp. Ther.*, **127**, 146–149.
Ambache, N. & Edwards, J. (1951). *Brit. J. Pharmacol.*, **6**, 311–317.
Ambache, N. & Lessin, A. W. (1955). *J. Physiol.*, **127**, 449–478.
Black, J. W. & Stephenson, J. S. (1962). *Lancet*, **2**, 311–314.
Brownlee, G. & Harry, J. (1963). *Brit. J. Pharmacol.*, **21**, 544–554.
Brownlee, G. & Johnson, E. S. (1963). *Ibid.*, **21**, 306–322.
Bucknell, A. & Whitney, B. (1964). *Ibid.*, **23**, 164–175.
Day, M. & Vane, J. R. (1963). *Ibid.*, **20**, 150–170.
Ellis, F. G., Kauntze, R., Nightingale, A. & Trounce, J. R. (1960). *Quart. J. Med.*, **29**, 305–315.
Fishlock, D. & Parks, A. G. (1963). *Brit. med. J.*, **2**, 666–667.
Gaddum, J. H. & Picarelli, Z. P. (1957). *Brit. J. Pharmacol.*, **12**, 323–328.
Gillespie, J. A. & Mackenna, B. R. (1960). *J. Physiol. (Lond.)*, **152**, 191–205.
Kosterlitz, H. W. & Lees, G. M. (1954). *Pharm. Rev.*, **16**, 301–339.
Levy, B. & Ahlquist, R. P. (1961). *J. Pharmacol.*, **133**, 202–210.
Trounce, J. R. & Nightingale, A. (1960). *Arch. Dis. Childh.*, **35**, 375–377.
Walder, D. N. (1953). *J. Physiol. (Lond.)*, **120**, 365–372.