J. Pharm. Pharmac., 1966, **18**, 265–270 Received October 22, 1965

The electrically stimulated ileum of the guinea-pig for measuring acetylcholine antagonism at different sites

G. D. H. LEACH

The transmurally stimulated guinea-pig ileum preparation was used to determine quantitatively the antagonism developed by hexamethonium and atropine against the emptying reaction and the longitudinal muscle response. Hexamethonium in concentrations of 1·5–6·0 μg/ml blocked the emptying reaction but larger doses, 8–10 μg/ml, failed to depress the longitudinal response to less than 50% of its original height. Atropine, on the other hand, in concentrations of 0·001–0·02 μg/ml, reduced the longitudinal response without affecting the emptying reaction. Thus, the preparation discriminates between acetylcholine antagonists acting at either the nicotinic or muscarinic site.

SINGLE or repeated electrical shocks applied across the wall of the guinea-pig ileum (Paton, 1955) produce a characteristic "twitch" or contraction of the longitudinal muscle in the undistended preparation which can be abolished by atropine in concentrations of 0·02 μg/ml. When the intraluminal pressure is raised to 1·5–3·0 cm of water, transmural stimulation has the effect of producing a co-ordinated movement of the circular and longitudinal muscles, termed an emptying reaction, which can be antagonised by ganglion blocking drugs. If the antagonism exhibited by atropine against the twitch response, and that of ganglion blocking drugs against the emptying reaction can be shown to be reasonably specific for their respective sites of action, the preparation would seem to be a convenient one for determining the relative intensity of action of acetylcholine antagonists at the muscarinic and nicotinic cholinergic sites. The quantitative effects are reported for atropine and hexamethonium in antagonising the responses of the transmurally stimulated guinea-pig ileum.

Methods

Adult guinea-pigs of either sex, 300–500 g, were killed and 3–5 cm of ileum removed from the small intestine 20 cm proximal to the ileo-caecal junction. This was set up in 10 ml of Krebs solution containing neostigmine 0·025 μg/ml at 34°, in the manner of a modified Trendelenburg preparation (Fig. 1).

Longitudinal muscle movements were isometrically recorded on smoked paper using a photoelectric method (Bell & Robson, 1936–37) with a mirror attached at the fulcrum of the lever; intraluminal pressure changes associated with either the emptying reaction or peristaltic reflex were measured with a polythene float recorder (Leach, 1958). The preparation was transmurally stimulated with platinum electrodes (Paton, 1956).

Transmurally elicited longitudinal contractions of the undistended preparation were obtained at 2 min intervals with a frequency of 1 shock/sec for 10 sec and a pulse duration of 0·1 msec. To ensure that

From the Pharmacological Laboratories, Department of Pharmacy, Bradford Institute of Technology, Bradford, 7.

the intraluminal pressures required to produce the emptying reaction (E) and the peristaltic reflex (P) remained constant throughout the experiment, two constant level fluid reservoirs, arranged at heights of 1·5–3·0 cm (E) and 6·0–8·0 cm (P) respectively (Fig. 1), above the fluid level of the organ bath, were used to distend the preparation. Transmural stimulation to elicit the emptying reaction was at a frequency of 1 shock/sec for 5 sec at a pulse duration of 0·03 msec.

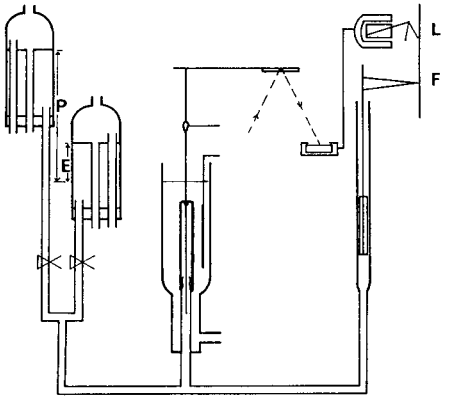

FIG. 1. Diagram of experimental arrangement. The ileum preparation suspended in a 10 ml bath is distended with Krebs solution from one of the two constant level reservoirs (E) and (P). Longitudinal muscle movements (L) are photo-electrically recorded on a smoked drum, and volume changes measured by the float recorder (F).

Longitudinal responses to transmural stimulation were obtained 1, 3, and 5 min after antagonist administration and emptying reactions at 7 and 9 min.

Estimates of antagonist potency against longitudinal responses were obtained by plotting response height, 5 min after the addition of the antagonist, as a percentage of the control height against log concentration of antagonist. The amount of antagonist required to reduce the response height by 50% could then be calculated. The difficulty in obtaining graded inhibition against the emptying reaction was overcome by expressing the antagonism to this response as the minimal concentration required to suppress two consecutive responses.

Results

The suitability of the preparation to discriminate between acetylcholine antagonists at the muscarinic and nicotinic site depended on tests with atropine and hexamethonium.

ATROPINE

Longitudinal response. Atropine, 0·001–0·02 μg/ml, reduced the longitudinal "twitch" muscle response (Fig. 2), the extent of the decrease depending upon the concentration of antagonist (Fig. 4).

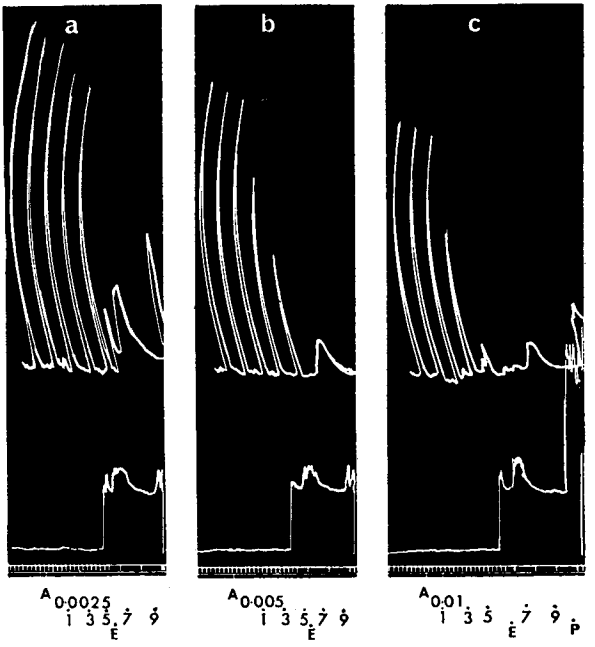

FIG. 2. Effect of atropine (A) on transmurally stimulated guinea-pig ileum. Upper record longitudinal responses; lower record intraluminal pressure. Atropine (a) 0·0025 μg/ml; (b) 0·005 μg/ml and (c) 0·01 μg/ml. 1, 3 and 5 refer to time in min after drug addition at which longitudinal responses were obtained; emptying reaction (E) elicited at 7 and 9 min after raising intraluminal pressure. Peristaltic reflex (P), Time = 30 sec.

TABLE 1. THE CONCENTRATION OF ANTAGONISTS REQUIRED TO INHIBIT THE LONGITUDINAL RESPONSE, THE EMPTYING REACTION AND THE PERISTALTIC REFLEX OF THE TRANSMURALLY STIMULATED GUINEA-PIG ILEUM

Exp. No.	Longitudinal response (calc. conc., μg/ml, producing 50% inhibition after 5 min)	Emptying reaction (conc., μg/ml, inhibiting two consecutive responses)	Peristaltic reflex (inhibitory conc., μg/ml)
(A) Atropine			
45	0·0046	>0·01	
46	0·0025	>0·01	
47	0·001	>0·01	
77	0·0047	0·06	<0·08
78	0·0046	0·04	0·04
79	0·0038	0·04	0·08
80	0·0024	0·04	0·06
Mean	0·0032	0·045 (Expts 77–80)	0·06 (Expts 78–80)
s.e.	0·0017 (n = 7)	0·016 (n = 4)	0·01 (n = 3)
(B) Hexamethonium			
18		1·5	
19		6·0	
20		2·0	
21		2·0	>8 0
22		4·0	
25		2·0	
26		2·0	>100·0
Mean		2·8	
s.e.		0·75 (n = 7)	
23	Contracture	130·0	
24	Contracture	320·0	

Emptying reaction. Atropine in concentrations needed to produce a 50% inhibition of the longitudinal response failed to suppress the emptying reaction; larger doses, 0·01–0·04 μg/ml, abolished the emptying reaction (Fig. 2).

Peristaltic reflex. An impairment of the peristaltic reflex was seen with doses of atropine, 0·04–0·08 μg/ml.

The mean concentrations of atropine needed to inhibit the longitudinal response and emptying reaction were 0·0032 μg/ml and 0·045 μg/ml respectively (Table 1).

HEXAMETHONIUM

Longitudinal response. Hexamethonium, 0·1–0·5 μg/ml, had no effect

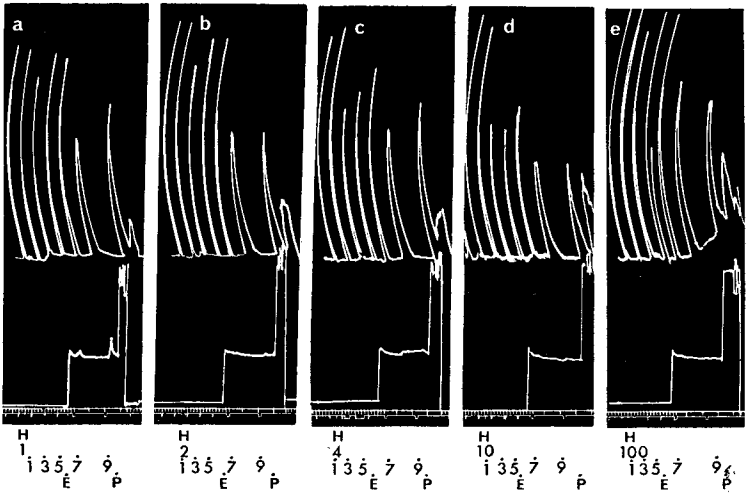

FIG. 3. Effect of hexamethonium (H) on transmurally stimulated guinea-pig ileum. Upper record longitudinal responses; lower record intraluminal pressure. Hexamethonium (a) 1·0 μg/ml; (b) 2·0 μg/ml; (c) 4·0 μg/ml; (d) 10 μg/ml and (e) 100 μg/ml. 1, 3 and 5 refer to time in minutes after drug addition at which longitudinal responses were obtained; emptying reaction (E) elicited 7 and 9 min after raising intraluminal pressure. Peristaltic reflex (P), Time = 30 sec.

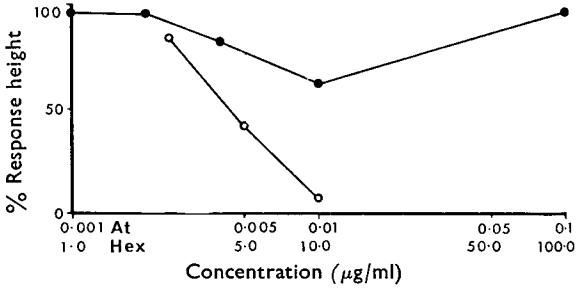

FIG. 4. Effect of hexamethonium and atropine concentrations on longitudinal response. Ordinate response height 5 min after drug addition as percentage of control. ●——● Hexamethonium (Hex), ○——○ Atropine (At). Calculated 50% inhibitory concentration of atropine in this experiment 0·0046 μg/ml.

on the longitudinal response. 0·5–3·0 μg/ml produced an initial decrease in the longitudinal response which was not sustained over the 5 min period of drug contact, and with the larger doses the 3 and 5 min stimulations were often seen to be greater than the initial control response (Figs 3 and 4).

The failure of hexamethonium to block the longitudinal response even in concentrations of 8 μg/ml or more, was not due to an inability of the preparation to respond to atropine-like compounds. In one experiment hexamethonium, 8 μg/ml, blocked the emptying reaction but the preceding longitudinal responses were unaffected; after washing, 0·01 μg/ml of propantheline reduced the longitudinal response to 35% of its initial height without affecting the emptying reaction.

Emptying reaction. Small doses of hexamethonium, 1·5–6·0 μg/ml, were able to suppress the emptying reaction (Fig. 3).

In two out of nine experiments, large doses of hexamethonium, 130–320 μg/ml, were required to inhibit the emptying reaction; the longitudinal response was not reduced, but was usually augmented; with the larger doses a contracture of the preparation was also seen. This unusual variation in sensitivity was not seen with other antagonists.

Peristaltic reflex. Although hexamethonium, 1·5–6·0 μg/ml, prevented the occurrence of an emptying reaction, increasing the intraluminal pressure to 7·5 cm water still produced a peristaltic reflex. In the preparations tested, hexamethonium, 100 μg/ml, did not impair the peristaltic reflex.

The mean concentration of hexamethonium needed to inhibit the emptying reaction was found to be 2·8 μg/ml; no value for longitudinal response inhibition could be determined for hexamethonium using this preparation (Table 1).

Discussion

In introducing the transmurally stimulated guinea-pig ileum preparation, Paton (1955) suggested that because of the sensitivity of the longitudinal twitch responses to atropine and the emptying reaction to ganglion blocking drugs, it is possible to "distinguish in a single preparation excitation of preganglionic, postganglionic . . . cell structures".

If these two responses of the preparation are indeed specific, then the preparation should prove useful not only in determining the type of cholinergic receptor involved in the antagonism, but also in providing quantitative information about the relative antagonistic potency at the muscarinic and nicotinic sites.

The results obtained in these experiments compare well with those quoted by Paton (1955, 1956) for this preparation. The mean concentration of atropine needed to produce a 50% inhibition of the longitudinal response was found to be 0·0032 μg/ml compared with 0·01–0·02 μg/ml quoted by Paton to abolish the longitudinal response; higher concentrations of 0·045 μg/ml affected the emptying reaction.

It may be seen from the results that although hexamethonium produces

an initial decrease in the longitudinal response, this is not sustained and with larger doses may even increase beyond the initial response height after 5 min contact. The appearance of stimulatory properties with respect to hexamethonium was reported by Feldberg (1951) and by Paton & Zaimis (1951), the latter authors ascribing the increased tone and rhythmic contractions to a release of vagal and sympathetic tone. Mantegazza, Tyler & Zaimis (1958), after summarising reported response enhancements to administered hexamethonium, considered the effect to be due to sensitisation of peripheral receptors. Increased vascular responses to 5-hydroxytryptamine and sympathomimetics after hexamethonium were also investigated by Laverty (1962).

However, the emptying reaction can be abolished with concentrations of 1·5–10·0 μg/ml of hexamethonium, even though the longitudinal muscle can still respond to stimulation. On the other hand, atropine in doses of 0·0032 μg/ml produced a 50% decrease in the longitudinal muscle responses to transmural stimulation, a concentration having no observable effect on the emptying reaction which was abolished only by larger doses of 0·01–0·04 μg/ml. The preparation would therefore appear to discriminate satisfactorily between those drugs which antagonise acetylcholine at its nicotinic and muscarinic sites of action.

One further point of interest is the extreme sensitivity of the emptying reaction to ganglion blocking drugs, as seen in Fig. 3, the emptying reaction being abolished with 8 μg/ml of hexamethonium, whilst increasing the intraluminal pressure still initiated a peristaltic reflex. Similar differences in the sensitivity of atropine were not seen, 0·04–0·08 μg/ml of atropine depressed both the emptying reaction and the peristaltic reflex.

Acknowledgement. I should like to express my grateful thanks to Professor G. Brownlee for his encouragement and advice during the course of this work.

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