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The action of angiotensin on the human colon *in vitro*

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Summary

1. The action of angiotensin on the circular and longitudinal muscle of the human colon has been studied *in vitro*. The ED50 value (g/ml) of circular muscle was $8{\cdot}7 \times 10^{-8}$ and of longitudinal muscle $3{\cdot}1 \times 10^{-8}$, while the corresponding figures for acetylcholine were $1{\cdot}6 \times 10^{-6}$ and $1{\cdot}1 \times 10^{-6}$, respectively. Angiotensin caused a sustained contraction associated with prolonged tachyphylaxis.

2. As the action of angiotensin was not antagonized by atropine, hexamethonium or procaine, angiotensin appeared to act directly on the smooth muscle of the human colon.

3. Lidoflazine is not a specific antagonist of angiotensin.

Introduction

Studies of the human colon *in vitro* have shown that the responses to drugs are different from those obtained in other animal preparations. The circular muscle was shown to relax in the presence of nicotine and choline phenyl ether (Fishlock & Parks, 1966a) and the taenia coli in the presence of dimethylphenylpiperazinium (DMPP) (Bucknell & Whitney, 1964). Both groups of investigators remarked on the absence of contractions in response to these nicotinic compounds. On the other hand, Wright & Shepherd (1966) obtained an inhibitory response with low concentrations of nicotine and DMPP but a contraction with higher concentrations. 5-Hydroxytryptamine also relaxes both the circular and the longitudinal muscle of the human colon *in vitro* (Fishlock & Parks, 1966b) and *in vivo* (Misiewicz, 1967). Bradykinin has an inhibitory effect on the human colon, although in high concentrations it contracts the taenia coli (Fishlock, 1966).

At present no substance is known which causes a contraction by an action on the nervous elements of the human colon. Because, from work with animal preparations, one would expect angiotensin to have such an indirect action (Ross, Ludden & Stone, 1960 ; Khairallah & Page, 1961 ; Robertson & Rubin, 1962), a study of the action of angiotensin on the human colon has been undertaken.

Methods

Longitudinal and circular muscle strips dissected free from the mucosa were removed from specimens of the distal half of the human colon, usually resected

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for carcinoma. The strips, 20–30 mm in length and 1–2 mm in width, were mounted in a 10 ml bath containing Krebs solution at 37° C gassed with 95% oxygen and 5% carbon dioxide.

The Krebs solution contained (mmol): Na^+ 140, K^+ 5·9, Ca^{2+} 2·5, Mg^{2+} 1·2, Cl^- 122, HCO_3^- 25, $H_2PO_4^-$ 1·2, SO_3^{2-} 1·2 and dextrose 11·5. The drugs were acetylcholine chloride, angiotensin II (Ciba), atropine sulphate, hexamethonium bromide, lidoflazine (Janssen Pharmaceuticals) and procaine hydrochloride. Concentrations refer to salts.

The contractions were recorded by means of an isotonic direct-writing lever on a smoked drum; the tension was 2 g and the magnification tenfold (Fishlock & Parks, 1966a). The contact time for acetylcholine was 20 s and for angiotensin 90 s.

Dose-response curves were obtained for acetylcholine and angiotensin on fifty-five strips of circular and twenty-four strips of longitudinal muscle. After initial exposure to an antagonist, the dose-response curves were repeated, the Krebs solution containing the antagonist throughout this period.

Atropine sulphate was used in a concentration of 10 μg/ml and the initial exposure time was 5 min. Hexamethonium bromide was used in a concentration of 20 μg/ml with an initial exposure time of 20 min. Procaine hydrochloride was used in a concentration of 50 μg/ml with an initial exposure time of 30 min. The preparation had only a single exposure to lidoflazine in a concentration of 500 ng/ml for 90 min, before repeating the dose response curves with Krebs solution free from antagonist.

Because angiotensin caused prolonged tachyphylaxis which, with high concentrations, persisted for as long as 40 min the intervals between additions of angiotensin were 50–60 min and frequent washouts were interspersed. For this reason the dose-response curves for angiotensin in each experiment were constructed from five or six single doses only.

Nicotine and DMPP were not used because their inhibitory effect on the colon would have added further difficulties to the experiments. The concentrations of hexamethonium bromide and procaine hydrochloride used, however, were sufficient to block the action of nicotine (Fishlock & Parks 1966a).

Results

Actions of angiotensin and acetylcholine

All longitudinal and circular muscle strips contracted in response to acetylcholine and angiotensin. The shape of the response varied considerably but it was not possible to differentiate between the fast and slow components which Godfraind, Kaba & Polster (1966) described for the guinea-pig ileum. The strips started to contract 15–30 s after the addition of angiotensin and maximum contractions were reached after 1–3 min. Although the bath fluid was frequently replaced with angiotensin-free Krebs solution, the strips relaxed slowly, reaching the original length after 10–15 min; longitudinal muscle strips usually took longer to relax than circular muscle strips. Figure 1 shows a typical response of the circular muscle to angiotensin.

Both circular and longitudinal muscle were more sensitive to angiotensin than to acetylcholine (Fig. 1 and Table 1). The mean ED50 values for the circular muscle

were $8{\cdot}7 \pm 0{\cdot}082 \times 10^{-8}$ g/ml ($n=40$) of angiotensin and $1{\cdot}6 \pm 0{\cdot}0009 \times 10^{-6}$ g/ml ($n=40$) of acetylcholine, while for the longitudinal muscle the mean ED50 values were $3{\cdot}1 \pm 0{\cdot}13 \times 10^{-8}$ g/ml ($n=22$) of angiotensin and $1{\cdot}1 \pm 0{\cdot}0014 \times 10^{-6}$ g/ml ($n=22$) of acetylcholine. Although angiotensin caused prolonged tachyphylaxis, there was no cross tachyphylaxis between angiotensin and acetylcholine.

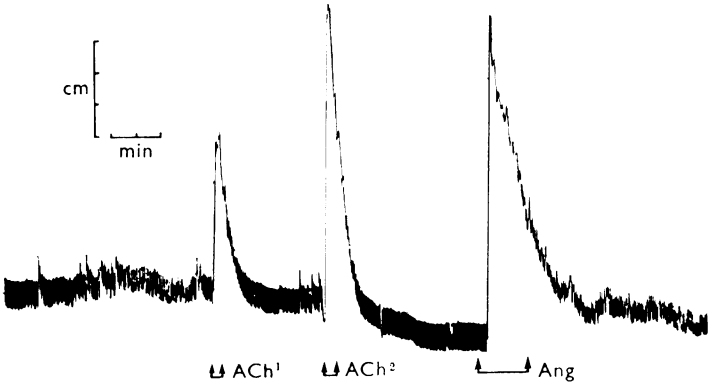

FIG. 1. Action of angiotensin on the human colon. Circular muscle strip. At Ang, angiotensin (50 ng/ml) was added to the bath; acetylcholine was added to the bath at ACh^1 (10 μg/ml) and at ACh^2 (50 μg/ml). Signals indicate contact time.

TABLE 1. *Effects of atropine, hexamethonium and lidoflazine on the responses to acetylcholine and angiotensin of circular muscle and longitudinal muscle of the human colon*

Drug	Concentration (μg/ml)	No. of experiments	Mean concentration ± S.E.M. of angiotensin giving 50% of maximum response (log ng/ml)	P	Mean concentration ± S.E.M. of angiotensin giving 50% of maximum response (log ng/ml)	P
(*a*) Circular muscle						
Atropine	0	7	1·41 ± 0·49	>0·6	2·73 ± 0·14	<0·001
	10	7	1·69 ± 0·44		6·17 ± 0·14	
Hexamethonium	0	6	1·77 ± 0·20	>0·1	2·57 ± 0·20	>0·7
	20	6	2·28 ± 0·30		2·66 ± 0·14	
Procaine	0	6	2·59 ± 0·37	>0·7	3·36 ± 0·24	>0·1
	50	6	2·77 ± 0·39		3·90 ± 0·17	
Lidoflazine	0	12	1·84 ± 0·14	<0·005	2·94 ± 0·14	<0·001
	0·5	12	2·53 ± 0·14		3·80 ± 0·14	
(*b*) Longitudinal muscle						
Atropine	0	8	1·26 ± 0·14	>0·5	2·78 ± 0·20	<0·001
	10	8	1·11 ± 0·17		5·94 ± 0·10	
Hexamethonium	0	6	1·95 ± 0·22	>0·9	3·96 ± 0·10	>0·4
	20	6	1·92 ± 0·17		4·13 ± 0·17	
Procaine	0	7	1·61 ± 0·20	>0·7	3·52 ± 0·24	>0·4
	50	7	1·73 ± 0·26		3·80 ± 0·24	

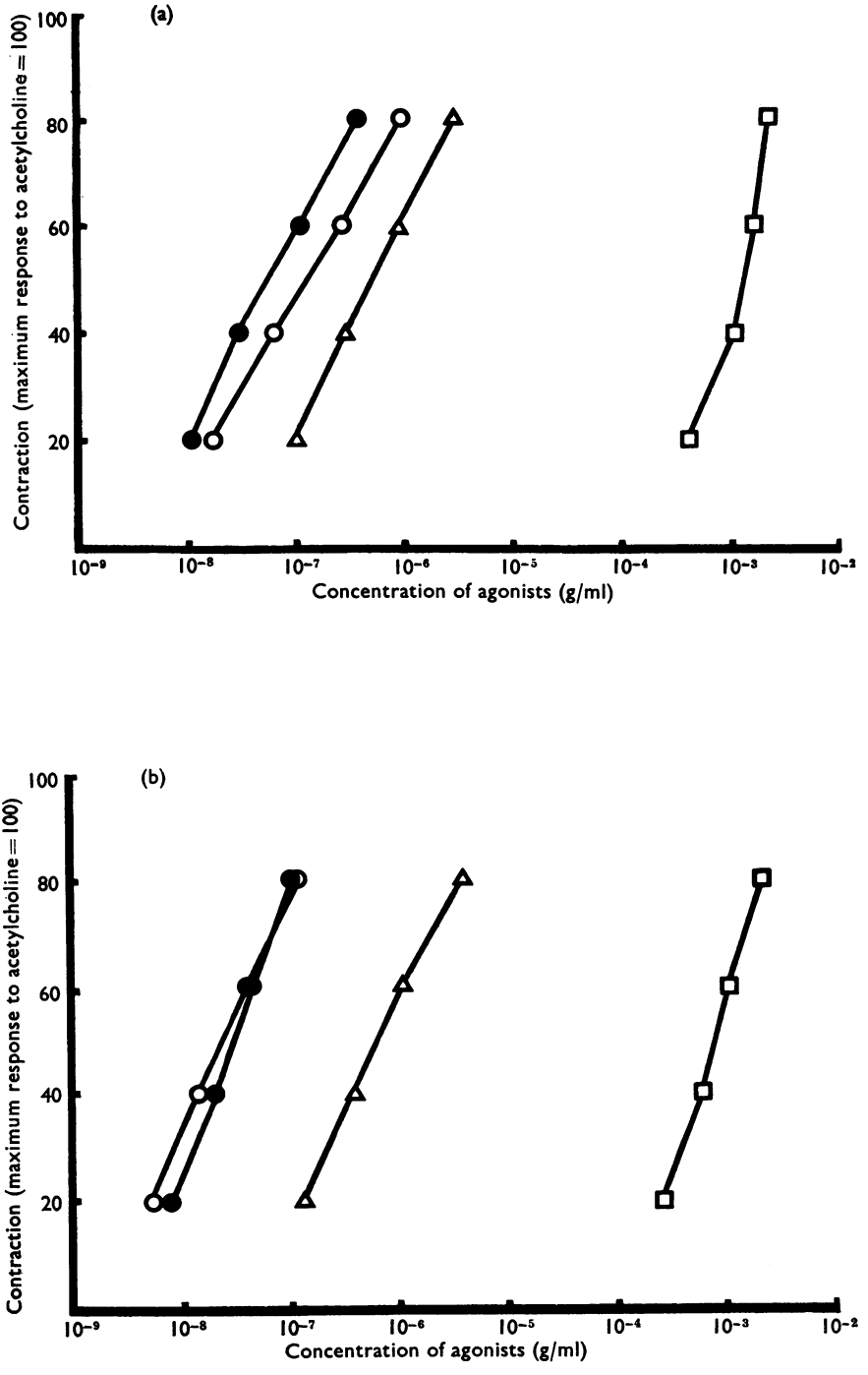

FIG. 2. Effect of atropine sulphate (10 μg/ml) on the response of circular (a) and longitudinal muscle (b) of the human colon to acetylcholine and angiotensin. Abscissa: Concentration of angiotensin and acetylcholine (g/ml) plotted logarithmically. Ordinate: % of maximum response to acetylcholine. Each curve represents the mean of seven (a) or eight (b) experiments. Acetylcholine; △——△ before and □——□ after exposure to atropine. Angiotensin; ●——● before and ○——○ after exposure to atropine.

Effects of procaine, hexamethonium and atropine

Procaine and hexamethonium had no significant effect on the responses of circular or longitudinal muscle to either agonist (Table 1). The responses of both circular and longitudinal muscle to acetylcholine were markedly reduced after exposure to atropine sulphate, but the responses to angiotensin were not affected. Figure 2 shows the mean dose-response curves of acetylcholine and angiotensin, for circular and longitudinal muscle, before and after exposure to atropine sulphate (10 μg/ml).

Effect of lidoflazine

In preliminary experiments it was found that a concentration of 50 ng/ml of lidoflazine applied for 90 min did not affect the response to either acetylcholine or angiotensin. Above this concentration, lidoflazine reduced the responses of the circular and longitudinal muscle to both agonists (Table 1), but there was no significant difference between the antagonist effects on the actions of angiotensin and acetylcholine ($P>0{\cdot}5$).

Discussion

Several workers have studied the effect of the octapeptide angiotensin II on the guinea-pig ileum. Khairallah & Page (1961) reported that atropine reduced the effect of angiotensin by 60–70% but did not abolish it. Later, they showed (Khairallah & Page, 1963) that the action of angiotensin was reduced by nicotine and DMPP and concluded that angiotensin had a direct action on smooth muscle and also an indirect action by stimulation of the ganglion cells of the myenteric plexus. Ross *et al.* (1960) found that the action of angiotensin on the guinea-pig ileum could be blocked by atropine, and postulated that angiotensin acted on the post-ganglionic cholinergic mechanism. Robertson & Rubin (1962), using rabbit and guinea-pig intestine, found that the effect of angiotensin was increased by the anti-cholinesterase BW284C57 but decreased by botulinum toxin.

Godfraind *et al.* (1966) found that angiotensin had a direct and an indirect action on the circular muscle of the guinea-pig ileum, but only a direct action on the longitudinal muscle. The indirect action was blocked by atropine and the direct action by lidoflazine. They concluded that lidoflazine was a specific antagonist of angiotensin.

The experiments described in this paper show evidence only for a direct action of angiotensin on the smooth cells of the longitudinal and circular layers of the human colon, as atropine, hexamethonium and procaine did not reduce the responses to angiotensin. Lidoflazine antagonized the actions of both angiotensin and acetylcholine on the circular muscle, so it is not a specific antagonist of angiotensin in this preparation.

The prolonged tachyphylaxis observed after exposure of the human colon *in vitro* to angiotensin may be of relatively little importance *in vivo* because of the presence of angiotensinase (Khairallah, Irvine, Bumpus & Turker, 1966).

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