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Inhibition by lidoflazine of the contractile response of the rat isolated colon to angiotensin

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1. In the rat isolated ascending colon, lidoflazine in a concentration of 5×10^{-7}M slightly enhanced the response to angiotensin, but in a concentration of 10^{-6}M it was a strong and unsurmountable antagonist.

2. Exposure to lidoflazine (10^{-6}M) also inhibited the contractile responses to acetylcholine, 5-hydroxytryptamine and barium ions. The antagonism of acetylcholine and 5-hydroxytryptamine was unsurmountable and quantitatively similar to that of angiotensin. The antagonism of barium ions was much weaker and probably competitive.

3. The inhibitory effect of papaverine (10^{-5}M) showed a pattern similar to that found for lidoflazine.

4. It is concluded that the antagonism of angiotensin by lidoflazine in the rat colon is non-specific.

Godfraind, Kaba & Polster (1966) have published evidence that lidoflazine, a drug which causes coronary vasodilation *in vivo* (Schaper, Xhonneux & Jageneau, 1965), is a potent, non-competitive inhibitor of the direct contractile effect of angiotensin on the smooth muscle of the guinea-pig ileum *in vitro*. In this preparation, lidoflazine also antagonizes the action of acetylcholine, histamine and bradykinin. It is most effective against angiotensin, however, so Godfraind *et al.* (1966) concluded that lidoflazine is a highly specific antagonist of the action of angiotensin on the smooth muscle of the guinea-pig ileum and suggested that this inhibitory relationship be investigated further on other preparations. We have done this using the isolated ascending colon of the rat, because Regoli & Vane (1964) have shown that, of all the preparations they investigated, the smooth muscle of the rat ascending colon was very sensitive to angiotensin but much less to other substances. Some of the results described in this paper have been reported to the American Society for Pharmacology and Experimental Therapeutics (Reit & Ellis, 1968).

Methods

Wistar rats of either sex weighing about 250 g were killed by stunning and then bleeding from the carotid arteries. The first 3 cm of ascending colon was removed,

washed and set up according to the procedure of Regoli & Vane (1964). The colon segment was suspended in a 10 ml. organ bath containing Krebs solution of the following composition (in g/l. of distilled water): NaCl 6.9, KCl 0.35, $CaCl_2 \cdot 2H_2O$ 0.37, KH_2PO_4 0.16, $MgSO_4 \cdot 7H_2O$ 0.29, glucose 1, $NaHCO_3$ 2.1. Pronethalol (1.9×10^{-6}M) was added to the Krebs solution because Regoli & Vane (1964) found that this drug reduces the high spontaneous activity of the rat colon while decreasing only slightly its sensitivity to angiotensin. A mixture of 95% oxygen and 5% carbon dioxide was bubbled vigorously through the organ bath and the reservoir of solution.

The load on the colon segment was 2 g. Contractions were magnified 18-fold by means of a frontal writing isotonic lever and were recorded on smoked kymograph paper.

In all experiments agonists were injected into the bath in volumes of 0.1–0.3 ml. with an interval of at least 6 min between successive injections. Each dose was left in contact with the tissue for 90 sec, after which time the bath was rinsed out two or more times. After equilibration with the antagonist, which usually caused the colon segment to relax somewhat, the recording baseline was readjusted to the control level.

A stock solution of lidoflazine (10^{-2}M) was prepared by dissolving it in 0.4 M acetic acid ; 0.05 or 0.1 ml. of this solution was added to 1 l. of the Krebs solution. In control experiments addition of 0.1 ml. of 0.4 M acetic acid to 1 l. of Krebs solution did not alter the responsiveness of the colon segment.

The following substances were used: angiotensin (val^5-Hypertensin II-asp-beta-amide, Ciba), acetylcholine bromide, 5-hydroxytryptamine creatinine sulphate (5-HT), barium chloride, lidoflazine (Janssen), papaverine hydrochloride and pronethalol (Alderlin, Ayerst). Concentrations are expressed as M.

Results

Lidoflazine-angiotensin antagonism

When lidoflazine (10^{-6}M) was added to the fluid bathing an isolated segment of rat colon, three effects were observed: within 1 min the colon segment relaxed and its spontaneous movements decreased in amplitude ; after 5 min the contractile response to angiotensin (9.6×10^{-9}M) was depressed by about 70% (Fig. 1). During 90 min exposure to lidoflazine there was little further decrease in the responsiveness to angiotensin. When the colon was bathed again in lidoflazine-free Krebs solution, there was little change in resting tone although the spontaneous movements, which had begun to recover while lidoflazine was still present, increased in amplitude at a more rapid rate. In this experiment, recovery of the responsiveness of the colon to angiotensin was minimal and very slow.

The effect of lidoflazine on the responses of five colon segments to angiotensin (9.6×10^{-9}M) is shown in Fig. 2. The inhibition was rapid in onset. Five minutes after addition of lidoflazine to the bath fluid the mean response to angiotensin was 57% of the mean control response. Maximal inhibition was attained more gradually during the next 45 min when the mean response fell to 41% of the control. Therefore, in order to obtain maximal inhibition in subsequent experiments, the colon segments were exposed to lidoflazine for 1 hr. After changing back to the lidoflazine-free Krebs solution, the recovery of responsiveness to angiotensin was

slow and incomplete in three out of five experiments ; after the preparations had been bathed in lidoflazine-free Krebs solution for 65 min, their responses to angiotensin were only 11, 42 and 50% of the mean control response. In the other two experiments, however, recovery was virtually complete within 35 min of changing over to the lidoflazine-free solution. This great variability accounts for the relatively large standard errors of the last four mean values shown in Fig. 2. The reason for this variability was not determined.

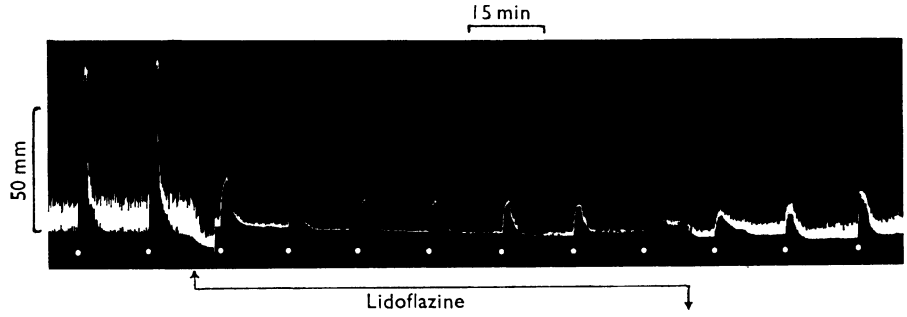

FIG. 1. Continuous record of movements of rat colon preparation bathed in Krebs solution at 37° C. At the white dots, injections of angiotensin (9.6×10^{-12} moles/ml.) into the bath. Baseline readjusted before third injection. Between the arrows, lidoflazine (10^{-6}M) was present in the Krebs solution. Time calibration, 15 min. Vertical scale, 50 mm.

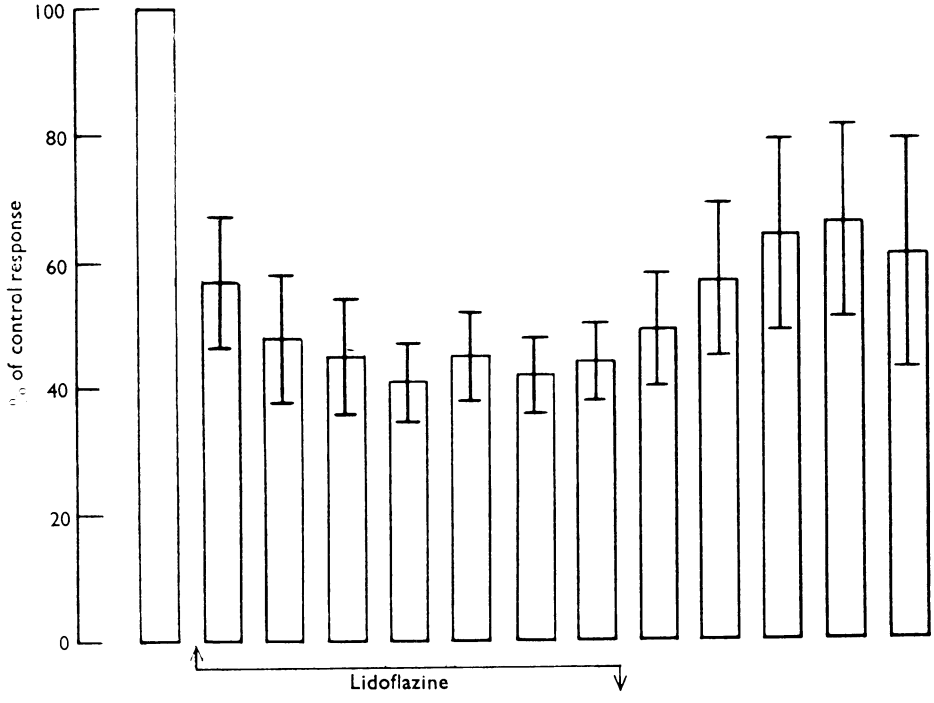

FIG. 2. Effect of lidoflazine on the responses of five rat colon preparations to angiotensin. In each experiment, angiotensin (9.6×10^{-12} moles/ml.) was injected into the bath at intervals of 15 min (see Fig. 1). Columns represent means, and bars standard errors, of responses elicited at the same relative times in each experiment and expressed as % of control (100% = 69.8 ± 9.5 mm). Between the arrows, lidoflazine (10^{-6}M) was present in the Krebs solution, from 5 min before the responses indicated by the second column until 5 min after the responses indicated by the eighth column.

Effects of lidoflazine on other agonists

In order to assess the nature and specificity of the antagonist action of lidoflazine, a comparison was made of the effects of lidoflazine on the dose-response curves for angiotensin, acetylcholine, 5-HT and barium ions. In preliminary experiments the effects of lidoflazine on angiotensin and acetylcholine were compared on the same colon segment (Fig. 3). In a concentration of 10^{-6}M, lidoflazine was a potent antagonist of acetylcholine as well as of angiotensin ; but in a concentration of 5×10^{-7}M it caused only a slight inhibition of the responses to acetylcholine and actually enhanced those to angiotensin. This potentiating effect, which was observed in each of three experiments, was not investigated further.

Pairs of dose response curves for each of the four agonists, before and after the addition of lidoflazine to the bath fluid, were obtained on separate segments of colon (Fig. 4). The control curves confirmed the finding of Regoli & Vane (1964)

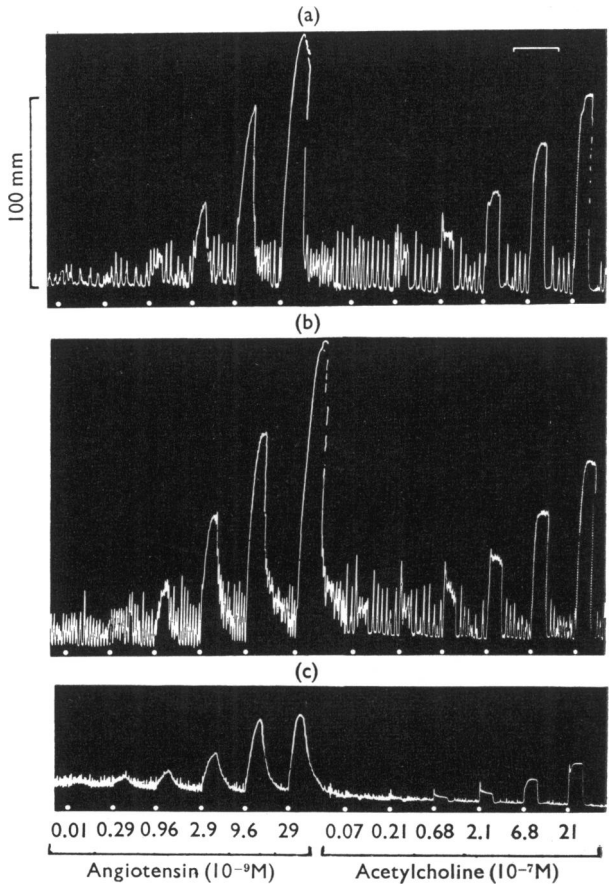

FIG. 3. Effects of lidoflazine on the responses of the rat colon to angiotensin and acetylcholine. At the white dots, injections into the bath of angiotensin on the left and of acetylcholine on the right. In each panel after the last injection of angiotensin the drum was stopped for 15 min. Panel (a), control responses ; panel (b), after 1 hr exposure to lidoflazine (5×10^{-7}M) ; panel (c), after 1 hr exposure to lidoflazine (10^{-6}M). Time calibration, 6 min. Vertical scale, 100 mm.

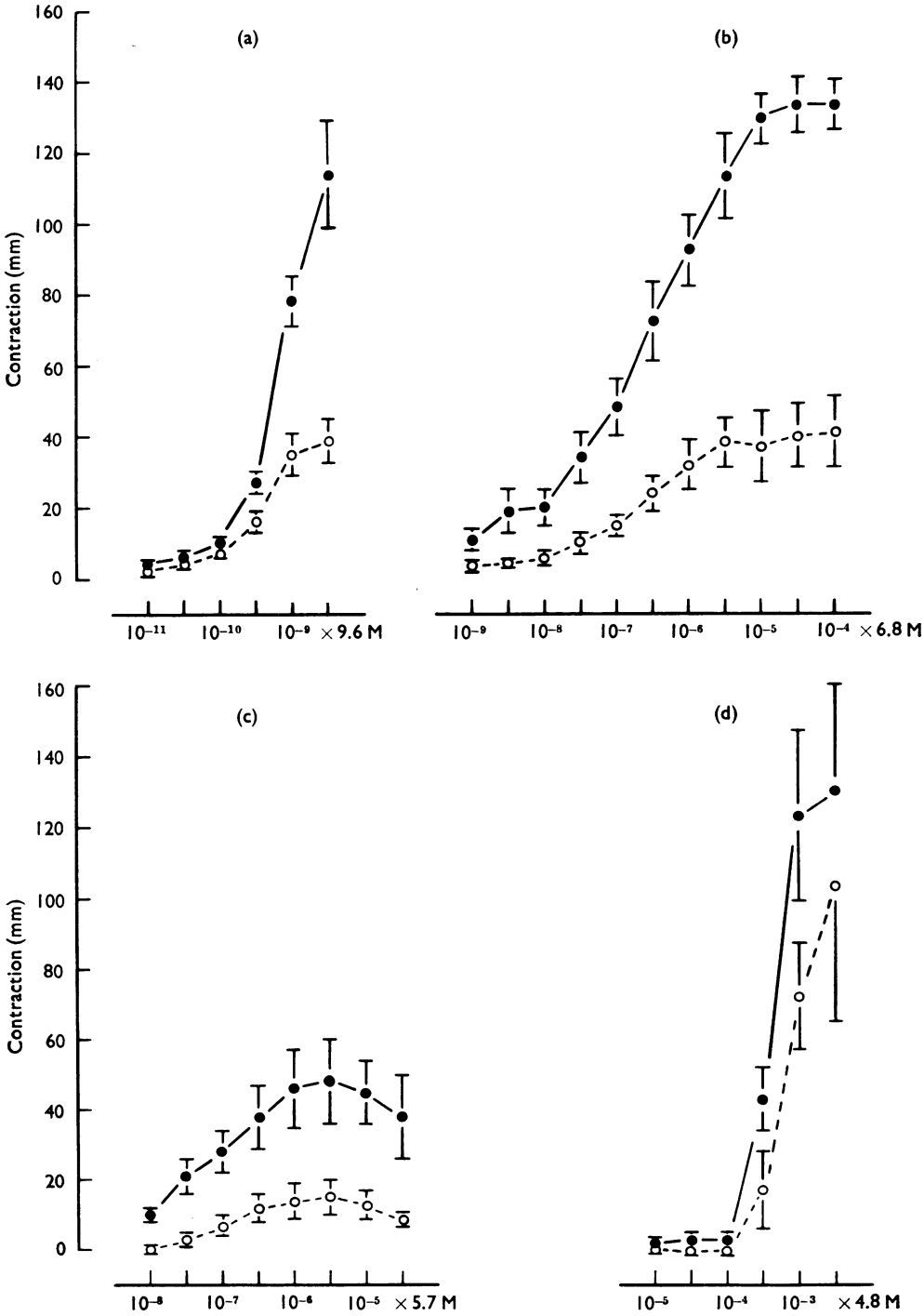

FIG. 4. Effect of lidoflazine on dose-response curves for angiotensin (nine colon preparations) in (a), acetylcholine (six preparations) in (b), 5-hydroxytryptamine (six preparations) in (c) and barium chloride (five preparations) in (d). Responses were elicited before (●——●) and after (○- - -○) 1 hr exposure to lidoflazine (10^{-6}M). Each point is the mean of at least three experiments; vertical bars represent standard errors of means. Ordinates: contraction height expressed in mm on the drum. Abscissae: molar concentrations on a logarithmic scale.

that the rat ascending colon is more sensitive to angiotensin than to acetylcholine or 5-HT ; they demonstrated further that this preparation is several orders of magnitude more sensitive to angiotensin than to barium ions. The ED50 values were approximately 5×10^{-9}M for angiotensin, 1.5×10^{-6}M for acetylcholine, 3×10^{-7}M for 5-HT and 2×10^{-3}M for barium ions.

Lidoflazine appeared to depress the dose-response curves for angiotensin, acetylcholine and 5-HT in the manner of an unsurmountable antagonist. The largest response to angiotensin on the control curve, if not actually maximal, was very nearly so. The degree of depression of the maximum by lidoflazine could therefore be assumed to be approximately 66%. The maximum response to acetylcholine was depressed by 69% and that to 5-HT by 68%. Thus, qualitatively and quantitatively, the lidoflazine-induced inhibition of the responses to these three substances was very similar. In contrast, lidoflazine caused the dose-response curve for barium chloride to undergo a slight parallel shift to the right, apparently without significant depression of the maximum, a finding which suggests competitive antagonism. Concentrations of barium chloride higher than 1.4×10^{-2}M were not used because even with this concentration a precipitate formed in the bath fluid.

Antagonism by papaverine

In view of the apparently unspecific nature of the antagonism produced by lidoflazine, we compared its action with that of papaverine, a drug well known for its ability to antagonize non-specifically a wide variety of substances causing contractions of other smooth muscle preparations. On addition of papaverine (10^{-5}M) to the bath, the colon segment abruptly relaxed and temporarily ceased its spontaneous movements, which were resumed about 5 min later with little decrease in amplitude but at a much lower frequency (Fig. 5). At the same time, the contractile response to angiotensin (9.6×10^{-8}M) was depressed by 79%. In the continuing presence of papaverine, the responses decreased a little further, then increased very gradually, and finally reached 41% of the control size. When the bathing fluid was changed to papaverine-free Krebs solution, no spontaneous movements occurred

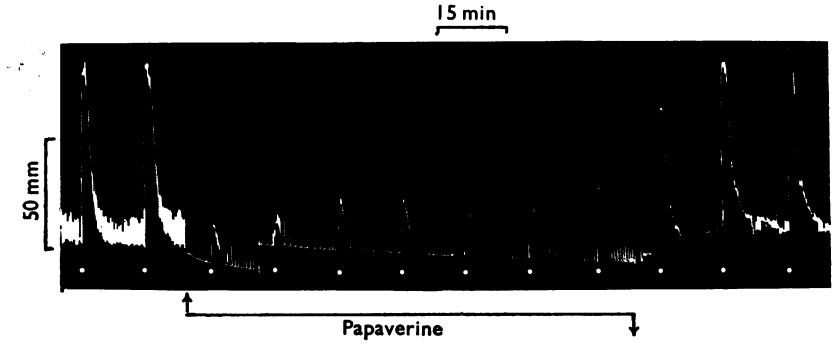

FIG. 5. Continuous record of movements of rat colon preparation bathed in Krebs solution at 37° C. At the white dots, injections of angiotensin (9.6×10^{-12} moles/ml.) into the bath. Baseline readjusted before fourth injection. Between the arrows, papaverine (10^{-5}M) was present in the Krebs solution. Time calibration, 15 min. Vertical scale, 50 mm.

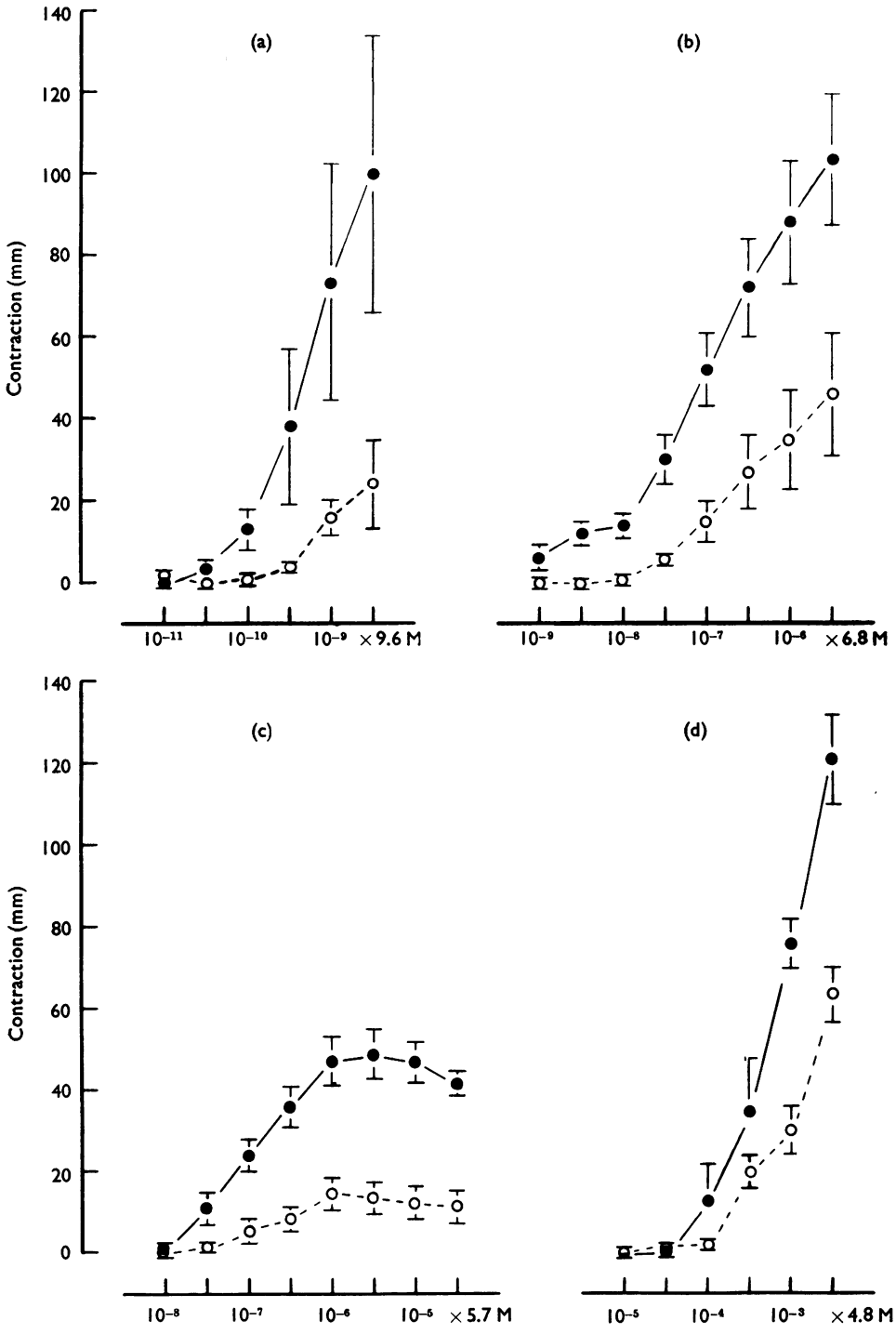

FIG. 6. Effect of papaverine on dose-response curves for angiotensin (a), acetylcholine (b), 5-hydroxytryptamine (c) and barium chloride (d). Each agonist was studied on four colon preparations. Responses were elicited before (●——●) and after (○- - -○) 1 hr exposure to papaverine (10^{-5}M). Each point is the mean of at least three experiments; vertical bars represent standard errors of means. Ordinates: contraction height expressed in mm on the drum. Abscissae: molar concentrations on a logarithmic scale.

during the first minute, but afterwards the movements were resumed with increasing frequency accompanied by an increase in tone and a very rapid recovery of responsiveness to angiotensin. Responses equal to the control size were obtained 20 min after the papaverine had been washed out. Similar results were obtained with three other colon segments.

In order to complete the comparison of the actions of papaverine and lidoflazine, dose-response curves were constructed for angiotensin, acetylcholine, 5-HT and barium chloride, before and after exposure of the colon to paraverine for 1 hr. In preliminary experiments, three concentrations of papaverine, 10^{-6}M, 5×10^{-6}M and 10^{-5}M, were tested against angiotensin and acetylcholine and it was found that 10^{-5}M papaverine was approximately as potent as 10^{-6}M lidoflazine ; therefore, a concentration of 10^{-5}M was chosen. The effects of papaverine on the dose-response curves obtained with the four agonists were remarkably similar to the effects of lidoflazine (Figs. 4 and 6). Thus, papaverine depressed the maximum responses to angiotensin and 5-HT to almost the same extent, namely 75% and 70%, respectively. It also depressed the responses to acetylcholine, but the maximum responses to this agonist in the presence and absence of papaverine were not obtained. Finally, the effect of papaverine on the dose-response curve for barium ions was similar to that of lidoflazine in that there was a small parallel shift to the right, a finding which suggests competitive inhibition.

Discussion

The results of this investigation show that lidoflazine is a fairly potent, unsurmountable antagonist of angiotensin on the rat ascending colon just as Godfraind *et al.* (1966) reported it to be on the guinea-pig ileum. Moreover, our results support the conclusion of these authors that lidoflazine antagonizes the direct action of angiotensin on the intestinal smooth muscle because, in the colon, angiotensin seems to act only on the smooth muscle cells directly (Regoli & Vane, 1964) and not also indirectly, as it does on the ileum through stimulation of the intrinsic cholinergic nerve supply (Khairallah & Page, 1961). We have confirmed the finding of Regoli & Vane (1964) that hyoscine does not affect the responses of the colon to angiotensin and, in addition, observed (Ellis & Reit, unpublished results) that the responses of the colon to angiotensin are not decreased by tetrodotoxin 10^{-7} g/ml., a concentration sufficient to abolish nerve-mediated responses of other innervated smooth muscle preparations *in vitro* (Gershon, 1967).

Godfraind *et al.* (1966) observed an enhancing effect of lidoflazine on the fast component of the contractile response of the guinea-pig ileum to angiotensin. This fast component, which they thought was due to the indirect action of the peptide, was potentiated by concentrations of lidoflazine lower than 10^{-7}M and inhibited by higher concentrations. The slow component of the response of the ileum to angiotensin, which they equated with the direct action of the peptide, was not potentiated by lidoflazine ; even a concentration as low as 10^{-9}M produced inhibition. Thus, the potentiating effect of 5×10^{-7}M lidoflazine on the responses of the rat colon to angiotensin is different from the potentiation of the indirect effect of angiotensin in the guinea-pig ileum, for in the rat colon angiotensin acts only directly on the smooth muscle.

In the rat colon, papaverine is an insurmountable antagonist of angiotensin. Papaverine differed from lidoflazine in that it diminished the frequency rather than

the amplitude of the spontaneous movements of the colon. Furthermore, the inhibitory effect of papaverine reached its peak sooner and was more readily and completely reversed by washing the colon with fresh Krebs solution. Yet, despite these differences, papaverine resembled lidoflazine in two striking respects. First, papaverine, like lidoflazine, inhibited the responses to angiotensin and 5-HT to about the same extent; thus neither antagonist was specific for angiotensin. Second, both papaverine and lidoflazine antagonized the contractile effect of barium much less effectively than that of the three other agents. This pattern of inhibitory effectiveness suggests a possible similarity in the mechanisms of action of the two antagonists, but, perhaps even more, it reflects the different sites and modes of action of the agonists. In smooth muscle cells, angiotensin, acetylcholine and 5-HT are believed to interact with specific receptors in the cell membranes; barium ions, on the other hand, have relatively little affinity for such superficial sites and are thought to pass into the cells activating their contractile machinery from within (Daniel, 1964). It is also possible that barium ions, as they do elsewhere (Kosterlitz & Lees, 1964), excite neural rather than muscular structures in the rat colon, in contrast to the direct excitation of the muscle cells by the other three agents.

In conclusion, the specificity of the antagonism of angiotensin by lidoflazine observed in the guinea-pig ileum by Godfraind *et al.* (1966) was not found in the rat ascending colon, in which lidoflazine proved to be non-specific, for it inhibited with equal effectiveness responses to angiotensin, acetylcholine and 5-HT. In this respect lidoflazine resembled papaverine, a non-specific relaxant of smooth muscle. These findings agree with the observations of Turker & Kayaalp (1967), who have reported that, in the rat fundus and the rabbit aortic strip, lidoflazine was a much more effective antagonist of 5-HT than of angiotensin.

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