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SPECIFIC ANTAGONISM TO THE DIRECT AND INDIRECT ACTION OF ANGIOTENSIN ON ISOLATED GUINEA-PIG ILEUM

BY

T. GODFRAIND, A. KABA, AND P. POLSTER

From the Department of General Pharmacodynamics, University of Louvain, Louvain, Belgium

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In 1961 Khairallah & Page reported that angiotensin has a direct and an indirect action on the guinea-pig ileum. Their results were confirmed by Robertson & Rubin (1962). The part taken by the cholinergic nerves in this effect was demonstrated using atropine or ganglioplegic drugs. Nevertheless, no evidence was given about the relative importance of these two actions. This paper deals with the demonstration that these actions may be dissociated analysing the shape of the contractile response and its modification by atropine that mainly blocks the indirect action and lidoflazine that appears to be a specific antagonist of the direct action. Lidoflazine, a potent coronary vasodilator (Schaper, Xhonneux, & Jageneau, 1965), has been tested as an angiotensin antagonist by analogy with the action of cinnarizine (Schaper, Jageneau, Xhonneux, Van Nueten, & Janssen, 1963).

A preliminary communication on some of this work was made at the joint meeting of the Physiological Society and the Société Belge de Physiologie et Pharmacologie in Louvain in April, 1966.

METHODS

Guinea-pig ileum preparation

Pieces 4 to 5 cm long were cut from the terminal part of the guinea-pig ileum and suspended in a 50 ml. bath containing Tyrode solution at 37° C gassed with 95% O_2 and 5% CO_2.

The composition of Tyrode solution was as follows: m-mole, NaCl 137, KCl 2.68, $CaCl_2$ 1.82, $MgCl_2$ 0.105, NaH_2PO_4 0.417, $NaHCO_3$ 11.9, glucose 5.55.

Longitudinal smooth muscle preparation

The longitudinal muscle of the guinea-pig ileum was separated from the circular layer and the mucosa as described by Rang (1964). It was kept during 1 hr at 37° C in Krebs bicarbonate gassed with 95% O_2 and 5% CO_2, before the assay, which was performed in Tyrode solution.

The composition of Krebs bicarbonate was as follows: m-mole, NaCl 112, KCl 5, $NaHCO_3$ 25, KH_2PO_4 1, $MgSO_4$ 1.2, $CaCl_2$ 2.5, glucose 11.5. Such a procedure was followed because the restoration of the ionic content after dissection is more complete in Krebs (Paton & Rothschild, 1965) than in Tyrode solution (Godfraind, Godfraind-De Becker & Sprumont, 1965).

Recordings

Recordings were made by isotonic lever on a smoked drum or by isometric lever with two strain gauges as part of a balanced bridge, the output of which was fed into a RS Dynograph recorder or a Kipp Micrograph recorder. In all experiments, muscles were initially loaded with 1 g.

Calculation of results

The response of the preparations has been expressed as a percentage of the maximal response to histamine. The dose ratio has been measured at the 50% maximal response level for the agonist studied.

The action of the antagonists studied has been expressed in terms of pA_2 and pA_h according to Arunlakshana & Schild (1959).

Drugs

The following drugs were used: acetylcholine chloride, angiotensin (Hypertensine, Ciba), atropine sulphate, histamine chloride, bradykinin, and lidoflazine.

RESULTS

Shape of contractile response to angiotensin of isolated guinea-pig ileum and of longitudinal smooth muscle

The shape of the guinea-pig ileum isotonic response evoked by angiotensin depends on the dose of the agonist. Low doses (lower than 10^{-9}M) generally cause a progressive increase in contraction, the maximum of which is reached after 1 min 30 sec. With higher doses a composite response is obtained which may be divided into two parts: an initial and fast rise with a small and transient subsequent fall that we shall call the fast component and a second and progressive increase of contraction that we shall name the slow component (Figs. 1*a* and 7*a*). At very high doses (up to 5×10^{-7}M) fast oscillations are superimposed on the slow response; they appear irregularly and therefore leave it still possible to estimate the plateau value.

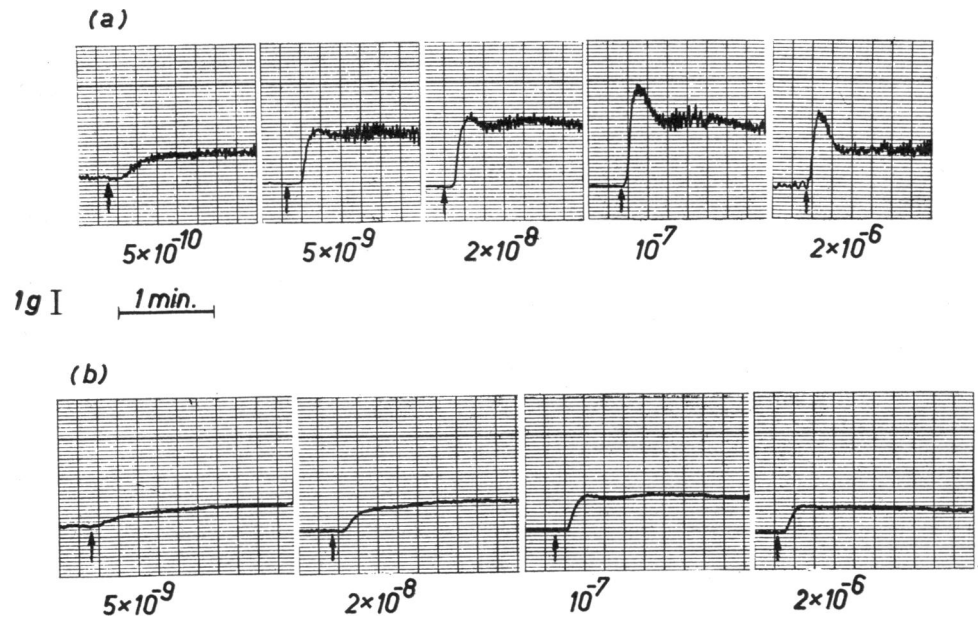

Fig. 1. Isometric records of response to angiotensin of guinea-pig ileum (*a*) and of longitudinal smooth muscle (*b*). Experiments were carried out in Tyrode solution at 37° C. Angiotensin was added to bath at ↑. Time at rest between injections was 10 min.

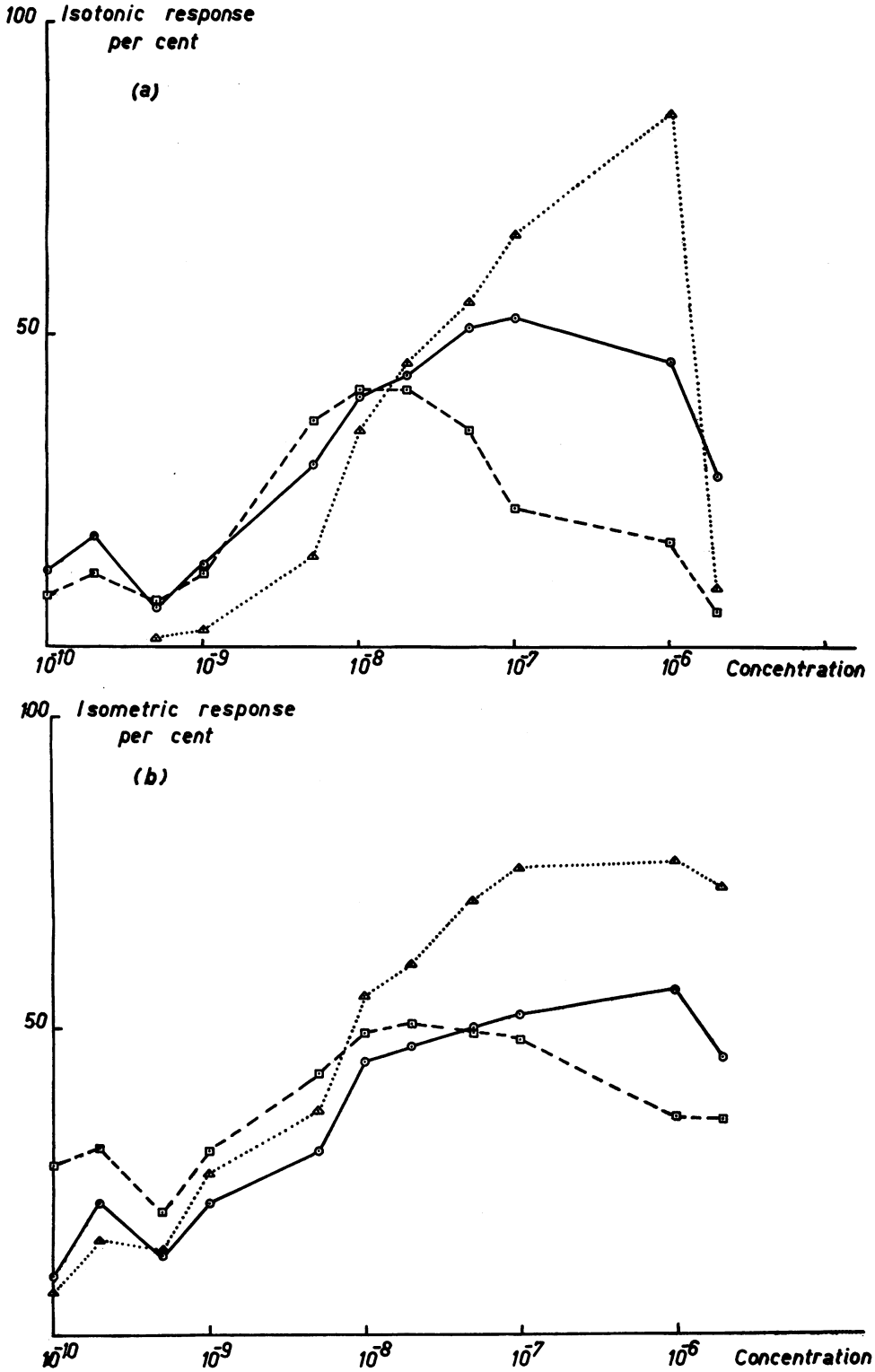

Fig. 2. Dose-effect curves of angiotensin on isolated guinea-pig ileum and on longitudinal smooth muscle. *a*=isotonic recordings; *b*=isometric recordings; Abscissa=molar concentration of angiotensin; Ordinate=response, in percentage of maximum response evoked by histamine of longitudinal smooth muscle (O) and of guinea-pig ileum measuring fast (△) or slow (□) component as illustrated in Fig. 4*a*. Angiotensin has been added every 12 min and removed after 90 sec. Each point is mean of at least 20 experiments, except points for concentrations lower than 10^{-9} and higher than 10^{-6}, which are means of at least five experiments.

As Fig. 2 illustrates, the relative magnitude of the slow and fast components is a function of the dose and depends on the conditions of recording: when the response is recorded isometrically the fast component is evoked by lower doses and the slow component does not fade as when recorded isotonically.

The optimum of the fast component attains 75 to 80% of the maximum response to histamine; on the other hand the slow component reaches only 50 to 55% of this same maximum response.

The response of the longitudinal smooth muscle to angiotensin presents only one slow component developing in 1 to 2 min (Fig. 1*b*). The dose-effect curve (Fig. 2) is a curve with an optimum equal to 55 to 60% of the maximum response evoked by histamine, the foot of which presents an irregular configuration due to the fact that angiotensin 2×10^{-10}M regularly evokes a response higher than that evoked by 5×10^{-10}M.

It is mainly at high doses that angiotensin causes tachyphylactic responses. This tachyphylaxis is reduced when there are sufficiently long periods at rest between injections. It seems to be due to a non-specific desensitization of the smooth muscle as the response to histamine is also reduced.

Action of atropine and eserine on contractile response to angiotensin of guinea-pig ileum and of longitudinal smooth muscle

The action of atropine on the two components of the contractile response to angiotensin and its suppression by eserine have been studied. In a first series of experiments, the action of the antagonist has been determined using one dose of angiotensin, generally 2×10^{-8}M. Such an experiment is illustrated by Fig. 3. It appears that atropine

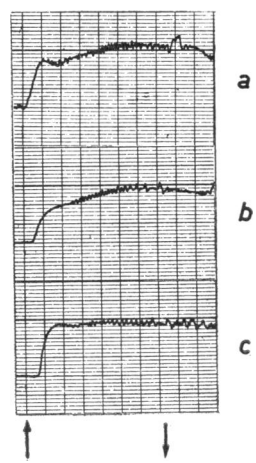

Fig. 3. Action of atropine and eserine on response of isolated guinea-pig ileum to angiotensin. Isometric response to angiotensin 2×10^{-8}M. Angiotensin was added to organ bath at ↑ and removed at ↓. a = Tyrode solution; b = Tyrode solution containing atropine 5×10^{-9}M, angiotensin was added after contact of 14 min with antagonist; c = 10 min later eserine 0.01 µg/ml. was added to the Tyrode containing atropine 5×10^{-9}M, and was allowed to act during 5 min before addition of agonist.

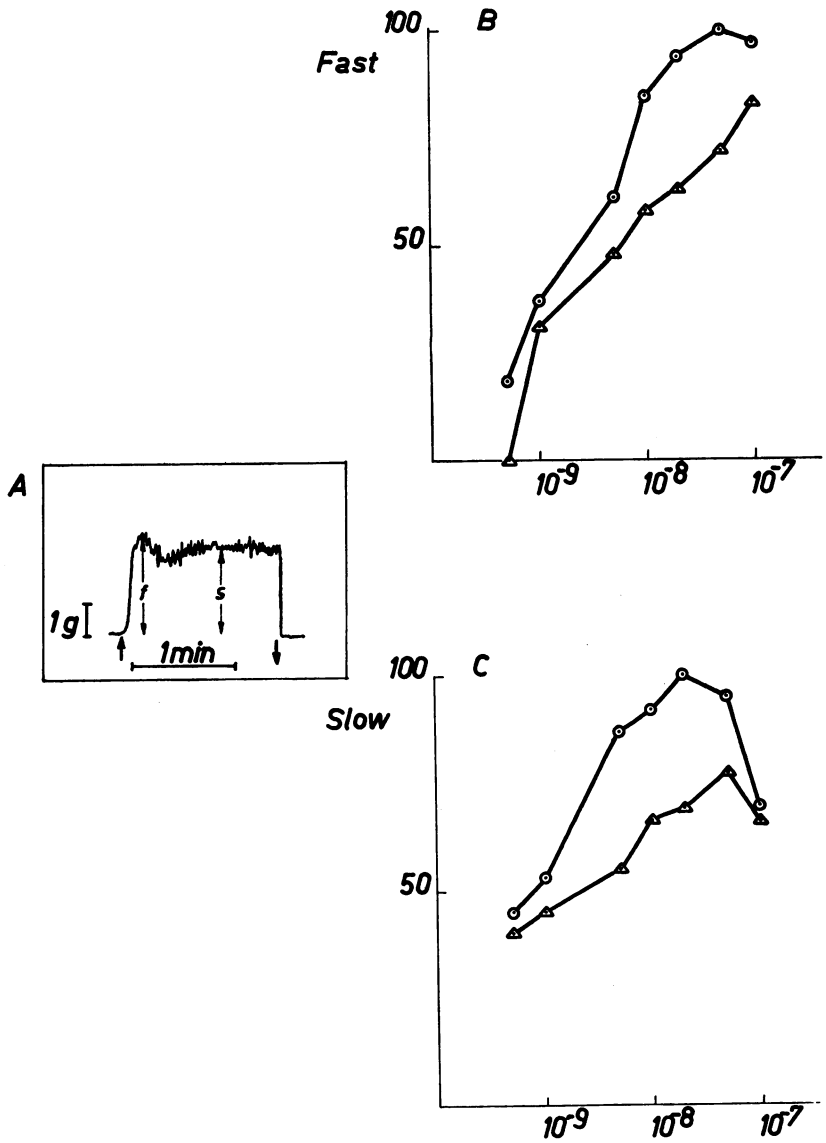

Fig. 4. Dose-effect curves of angiotensin on isolated guinea-pig ileum in absence and in presence of atropine 10^{-9}M. A=Isometric record of the response to angiotensin 5×10^{-8}M, with indications of mode of measuring fast component (f) and slow component (s). B=Dose-effect curves of fast component in absence (○) and in presence of atropine 10^{-9}M (△) 14 min after addition of antagonist. Ordinate=isometric tension in percentage of maximum tension of fast component. Abscissa=molar concentration of angiotensin. C=Dose-effect curve of slow component in absence (○) and in presence of atropine 10^{-9}M (△) 14 min after addition of antagonist. Ordinate=isometric tension in percentage of maximum tension of slow component. Abscissa=molar concentration of angiotensin.

5×10^{-9}M reduces the fast component of the contractile response without markedly modifying the slow component. The addition of eserine (0.01 μg/ml.) restores the fast component of the response.

In a second series of experiments three or six doses of angiotensin arranged between 5×10^{-10}M and 5×10^{-8}M were applied every 10 min during 1 min 30 sec in order to establish the dose-response curve. After 75 min at rest in Tyrode solution, atropine was added to the perfusion fluid, and after 14 min the sequence of doses of angiotensin was applied in the presence of atropine. In other experiments, atropine was kept in contact with the preparation for 90 min. By this procedure (Fig. 4) it was possible to determine dose ratios for the fast and the slow components. Values of log dose ratios measured at the 50% maximum response level are shown in Table 1.

Similar experiments have been performed with longitudinal smooth muscle ; log dose ratio values for atropine are also shown in Table 1.

TABLE 1

COMPARISON OF LOG DOSE RATIO VALUES MEASURED IN PRESENCE OF ATROPINE 10^{-9}M AND RELATED TO FAST AND SLOW ACTIONS OF ANGIOTENSIN

The number of experiments are given in brackets

Time of contact with antagonist (min)	Ileum fast	Ileum slow	Longitudinal slow
14	0·705 (4)	0·425 (4)	—
90	abolished (2)	0·730 (2)	0·788 (2)

Action of lidoflazine on contractile response to angiotensin

Preliminary assays were made in order to determine the time necessary to reach the equilibrium for the action of lidoflazine on the response of the guinea-pig ileum to angiotensin. They were performed with lidoflazine 5×10^{-7}M. It was observed that the slow component was more markedly reduced than the fast component and that the greatest effect was reached after a contact of 60 min.

The action of lidoflazine was established measuring the dose ratio and the reduction of the maximum response for the fast and slow components of the response of the guinea-pig ileum to angiotensin in the presence of various concentrations of the antagonist after a contact of 90 min. Six doses of angiotensin comprised between 5×10^{-10} and 5×10^{-8}M, applied every 10 min for 90 sec, were used in order to establish the dose-response curve.

The action of lidoflazine differs according to whether the fast or the slow component of the contractile response is considered. The fast component is potentiated by concentrations lower than 10^{-7}M and reduced by higher concentrations. The slow component is reduced by concentrations up to 10^{-9}M. Figure 5 shows log dose-effect curves of the slow component with lidoflazine obtained on the guinea-pig ileum. The slope of the curves becomes progressively flatter and their optima decline, but the dose that evokes the optimum response remains unchanged. Dose ratios have been measured at the 50% maximum response level, except with lidoflazine 10^{-7}M with which the dose ratio has been measured at the response nearest 50%, generally 45 to 47 per cent. Figure 6 illustrates the relation between the concentration of lidoflazine and the dose ratio. The slope of the line is 0.5. The intersection of the ordinate gives a pA_2 value of 9.3.

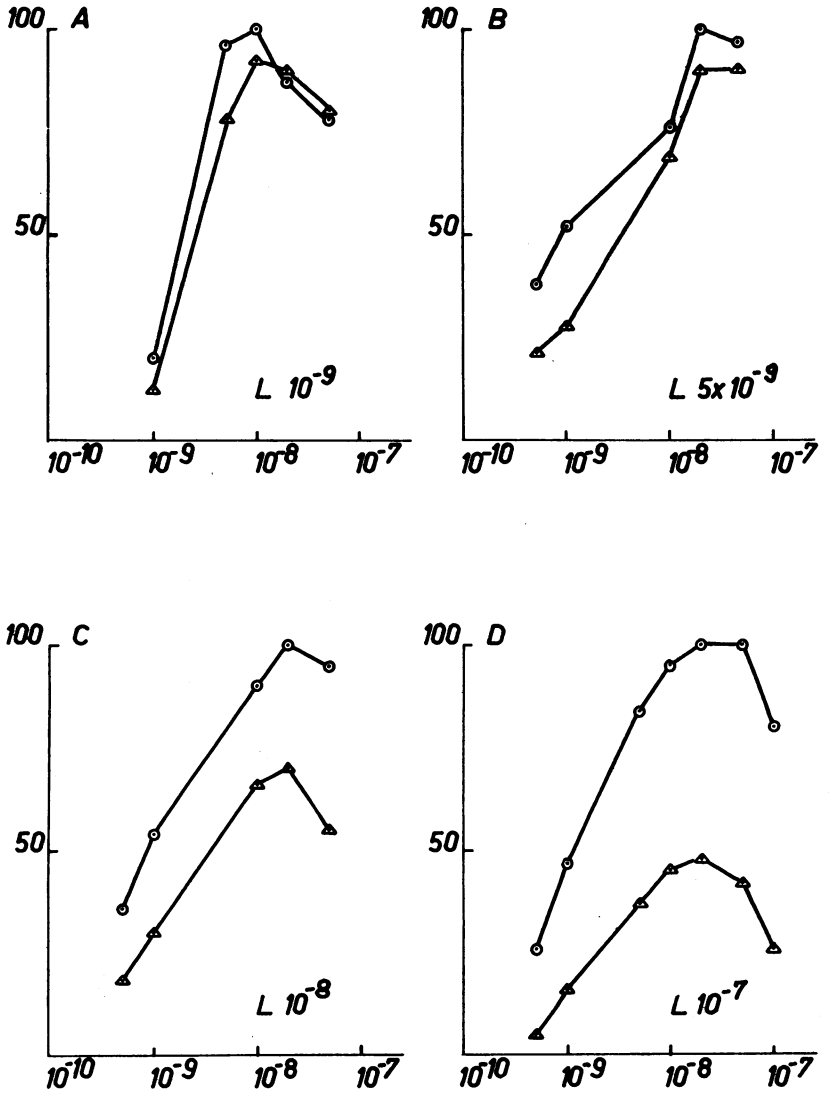

Fig. 5. Dose-effect curves of slow component of angiotensin with lidoflazine (L) at various molar concentrations as indicated on graph. A, B, C, D are different experiments ; A isotonic recording, B, C, D, isometric recording. Ordinate = response in percentage of maximum response of slow component in absence (○) and in presence of lidoflazine (△) 90 min after addition of antagonist. Abscissa = molar concentration of angiotensin.

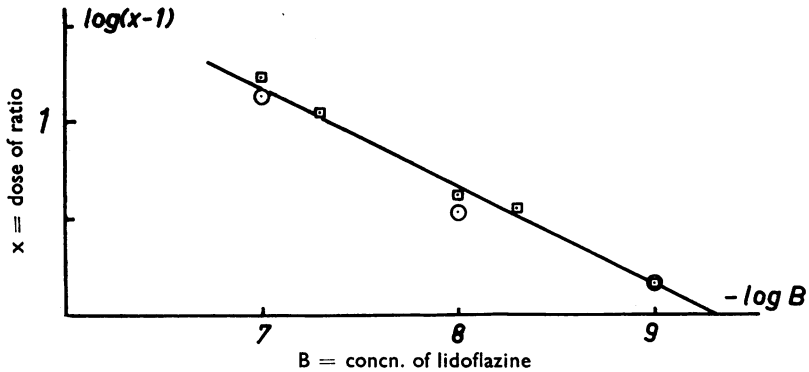

Fig. 6. Relation between concentration of lidoflazine and dose ratio of slow component of angiotensin action. The figure is a plot of equation: log $(x-1)=\log K_2-npA_x$ (Arunlakshana & Schild, 1959). Each point is mean of at least three experiments using guinea-pig ileum (□) or longitudinal smooth muscle (○) under isometric conditions. Contact with antagonist was 90 min.

Similar results have been obtained measuring the antagonism between lidoflazine and the action of angiotensin on the longitudinal smooth muscle: the pA_2 value is also equal to 9.3 (Fig. 4).

Specificity of lidoflazine as antagonist for slow component of angiotensin action

In order to obtain more information on the action of lidoflazine as an angiotensin antagonist, a series of experiments was performed using lidoflazine 10^{-7}M. The fast component of the response of the ileum to the peptide was measured isotonically and

TABLE 2

COMPARISON OF LOG DOSE RATIO VALUES MEASURED IN PRESENCE OF LIDOFLAZINE 10^{-7}M AND RELATED TO SLOW AND FAST ACTIONS OF ANGIOTENSIN AND ACTION OF ACETYLCHOLINE

The number of experiments are given in brackets. Contact of 90 min with the antagonist

Preparation	Agonist	Type of action	Log dose ratio
Ileum	angiotensin	slow	1·263 (4)
		fast	0·750 (5)
	acetylcholine	—	0·450 (2)
Longitudinal strips	angiotensin	slow	1·160 (6)
	acetylcholine	—	0·420 (2)

the slow component isometrically. The response of the ileum to acetlycholine and of the longitudinal smooth muscle to acetylcholine and angiotensin was measured isometrically. Dose ratios were established after a contact of 90 min. The results of these experiments are reported in Table 2. The highest log dose ratio value is that for the slow action of angiotensin.

Another illustration of the specific action of lidoflazine on the slow component of the guinea-pig ileum's response to angiotensin is given in the experiment shown in Fig. 7. In the presence of lidoflazine 2×10^{-7}M, the slow component is more reduced than the fast one (Fig. 7*b*); the addition of atropine 10^{-8}M is followed by a suppression of the fast component (Fig. 7*c*) which is partially restored by eserine (Fig. 7*d*).

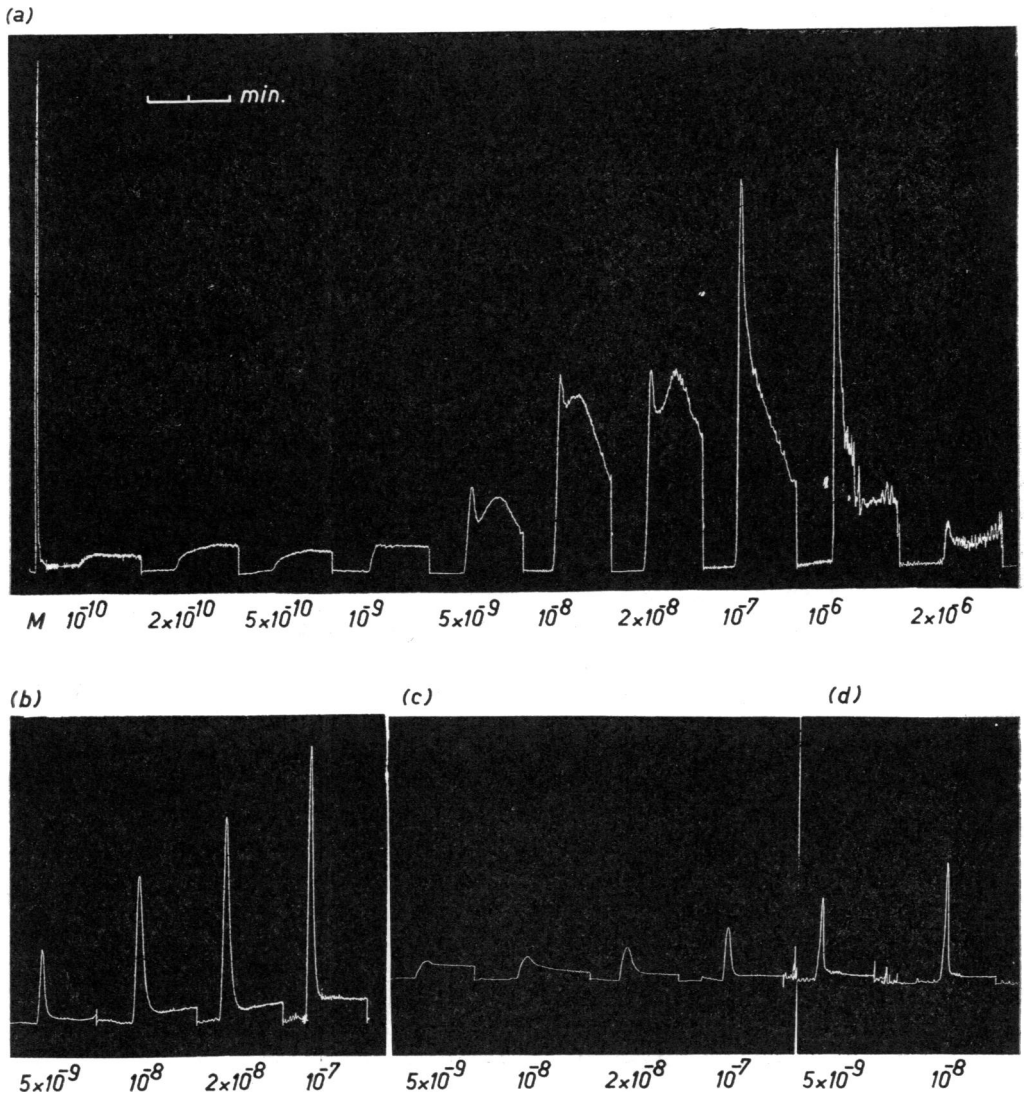

Fig. 7. Isotonic records of response of guinea-pig ileum to angiotensin, action of lidoflazine, atropine and eserine. The experiment was performed in sequence *a*, *b*, *c*, *d*. *a* = Response to angiotensin of guinea-pig ileum in Tyrode solution. M = maximum response to histamine ; the other records are responses to angiotensin. Experiment started 30 min after maximal contraction. Angiotensin added every 12 min and removed after 90 sec. *b* = Response in presence of lidoflazine 2×10^{-7}M, 90 min after addition of antagonist. *c* = Response in presence of lidoflazine 2×10^{-7}M plus atropine 10^{-8}M, added 14 min before assay. *d* = Response in presence of lidoflazine 2×10^{-7}M plus atropine 10^{-8}M and eserine 0.01 g/ml. added 5 min before assay.

The action of other agonists in the presence of lidoflazine has also been tested. The results are summarized in Table 3 which shows pA_2 and pA_h values with acetylcholine, histamine, bradykinin and angiotensin as agonist.

TABLE 3

THE pA_2 AND pA_h VALUES OF LIDOFLAZINE WITH VARIOUS AGONISTS

Agonist	pA_2	pA_h	pA_2–pA_h
Angiotensin slow	9·3	7·6	1·7
Angiotensin fast	7·85	6·82	1·03
Histamine	7·8	6·2	1·6
Acetylcholine	7·44	7	0·44
Bradykinin	7·14	6·46	0·68

DISCUSSION

Significance of the two components

The experimental results reported here show that the response of the guinea-pig ileum to angiotensin presents a fast and a slow component. The fast component is of minor importance with low doses of the peptide, and it is not present in the response of the longitudinal smooth muscle of the external layer of the ileum, which does not contain functional cholinergic fibres.

Atropine inhibits the fast component more than the slow one, and eserine overcomes the suppression of the fast component produced by atropine but does not affect the reduction of the slow component. Dose ratio values of atropine are similar as far as the inhibition of the slow component is concerned or that of the response of the longitudinal smooth muscle.

The pA_2 values of lidoflazine are the same as far as the slow component of the guinea-pig ileum and the action of angiotensin on the longitudinal smooth muscle are concerned. All these facts support the view that the fast component is due to the indirect action of angiotensin mediated through cholinergic nerves and that the slow component results from the direct action of the peptide on the smooth muscle.

Dose-effect curve of angiotensin

The analysis of the dose-effect curve of angiotensin shows that the maximal response produced by the peptide is always lower in our experimental conditions than that produced by histamine. The maximal response obtained is 70 to 80% of histamine for the indirect action and 50 to 60% for the direct action. The dose-effect curve of the direct action is a curve with an optimum that is characteristic of partial agonists. From such a curve we may predict that it will be difficult to find competitive antagonists of angiotensin using preparations of intestinal smooth muscle, as high doses of the peptide produce a tachyphylactic and fading response. It seems that the tachyphylaxis produced by angiotensin is due to a non-specific desensitization which should be caused by ionic modifications similar to those observed on isolated aorta (Daniel, 1965). The surprising configuration of the foot of the dose-effect curve may be due to tachyphylaxis, but more information is necessary in order to confirm this hypothesis.

A comparison of the dose-effect curves of the direct and indirect action recorded isotonically supports the view that the sensitivity of cholinergic nerves to the stimulating action of angiotensin is lower than that of the smooth muscle fibres. The dose that evokes the indirect action is always higher than 10^{-9}M; lower doses cause only a slow response.

The difference in shape of the dose-effect curves according to whether recordings are isometric or isotonic is similar to that found with other agonists (Paton & Rothschild, 1965). It may be attributed to a difference in the physical conditions of contractions due to levers. The consequence of this fact is that isometric recording appears to be more convenient in order to measure accurately both actions.

Action of antagonists

Experimental results show that atropine as well as lidoflazine are to be considered as angiotensin antagonists. Nevertheless, only lidoflazine is specific for the direct action of the peptide on intestinal smooth muscle. This suggests that specific peptidic receptors are located in tissues other than the rat colon studied by Regoli & Vane (1964).

As already explained, it appears that it is difficult, using guinea-pig ileum preparations for biological assays, to decide if an antagonist of angiotensin is competitive or not. As far as lidoflazine is concerned, none of Arunlakshana and Schild's criteria of a competitive antagonist can be applied. This would suggest that lidoflazine is a non-competitive but highly specific antagonist of angiotensin on the guinea-pig ileum. In order to clarify this matter further, it would be desirable to carry out experiments under other conditions and with other preparations.

SUMMARY

1. The contractile response of the guinea-pig ileum to angiotensin presents a fast and a slow component; the response of the longitudinal smooth muscle presents only a slow component.

2. Atropine abolishes the fast component and this action is overcome by eserine.

3. Lidoflazine is a non-competitive but highly specific antagonist for the slow action of angiotensin.

4. It is concluded that the fast component is due to the indirect action and the slow component to the direct action of angiotensin.

5. The significance of these results is discussed.

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