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THE ACTION OF ANGIOTENSIN II ON GUINEA-PIG ILEUM AND ITS MODIFICATION BY CHANGES IN SODIUM CONCENTRATION*

BY

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High Na^+ concentration in the bathing solution increases the contractile response of guinea-pig ileum to angiotensin II, whereas low Na^+ depresses the response (Blair-West & McKenzie, 1966). The response to acetylcholine (ACh) is depressed by both high and low Na^+. Hence, the potentiation of response to angiotensin is probably not due to the influence of high Na^+ on the response to ACh which is liberated by angiotensin. Khairallah & Page (1961, 1963) demonstrated that atropine (10^{-7} g/ml.) reduced without abolishing the contractile response of guinea-pig ileum to angiotensin. Blair-West & McKenzie (1966) showed that atropine at concentration of 10^{-8} g/ml. and higher reduced the response to angiotensin by approximately 60%, and totally abolished responses of equal magnitude to acetylcholine. Lewis & Reit (1966) have shown that angiotensin is a potent stimulant of autonomic ganglia. The results indicate that angiotensin acts at two or more sites in the tissue, one of which is a cholinergic pathway.

The present study was undertaken to determine the site or sites at which high Na^+ concentration potentiates the contractile response of guinea-pig ileum to angiotensin. The structures stimulated by angiotensin have been investigated using ganglion-blocking drugs, blockade of nervous conduction, depression of ACh release at junctions, and neuro-effector blockade by atropine. In each case the specificity of blockade has been assessed by comparing the effect on responses to angiotensin with those on responses to three other stimulating agents. Dimethylphenylpiperazinium (DMPP) was used to stimulate ganglion cells without significant direct action on the smooth muscle (Volle & Koelle, 1965). Single-shock electrical stimulation through extramural electrodes was considered, on the pubished evidence (Paton, 1955; Kern & Lembeck, 1959) to act principally on post-ganglionic axones. The evidence of Robertson & Rubin (1962) indicated that the contractile response of guinea-pig ileum to ACh is almost entirely accounted for by its stimulant effect on smooth muscle, with no significant contribution by action on the intramural nervous elements. This led to the use of ACh as a direct

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stimulant of smooth muscle but the results obtained suggest that it also stimulates indirectly.

The influence of high Na^+ concentration on responses to angiotensin in the presence of various blocking agents indicated that an increase of sodium concentration potentiates a direct action of angiotensin on smooth muscle cells.

METHODS

Segments of upper ileum, 2–3 cm in length, were taken from young male guinea-pigs killed by a blow to the neck. The preparations were suspended in a 20 ml. organ bath containing Krebs bicarbonate solution (NaCl, 6.9 ; KCl, 0.35 ; $CaCl_2.2H_2O$, 0.18 ; $NaHCO_3$, 2.1 ; $MgSO_4.7H_2O$, 0.29 ; KH_2PO_4, 0.16 ; glucose, 1 g/l.) bubbled with a 5% CO_2 in O_2 mixture and maintained at 37° C. " High sodium " media were prepared by adding weighed quantities of NaCl to the normal solution. " Low sodium " media were prepared by replacing the deficit of NaCl by an equi-osmolar quantity of sucrose.

Contractions were recorded with a force-displacement transducer coupled to an Offner dynograph. Earlier experiments used an approximately isometric method of recording, but in the majority a method basically equivalent to the auxotonic method described by Paton (1957) was adopted. In the former case, the intestinal segment was tied directly to the strain gauge, in the latter the segment was tied to the longer arm of a lever and the strain gauge was attached to the short arm close to the fulcrum. The two methods gave similar results, except that with auxotonic recording the preparation deteriorated less rapidly.

Contractile responses were measured at the maximal deviation from the baseline. No allowance was made for the variation of pattern of response with dose of stimulating agent. In this context variation of sodium concentration did not appear to modify selectively the " fast " or " slow " component of the response to angiotensin described by Godfraind, Kaba & Polster (1966).

Test drugs were prepared in Krebs solution and added to the bath in 0.1 ml. volumes at approximately 4 min intervals. Antagonists were added to the stock Krebs solution and were thus in continuous contact with the preparation.

Drugs used were angiotensin II (Asp^1-NH2-Val^5 angiotensin II ; Ciba) ; acetylcholine chloride (Roche) ; DMPP (1,1 dimethyl 4 phenyl piperazinium iodide ; Fluka) ; hemicholinium-3 (Aldrich) ; atropine sulphate (Drug Houses of Australia) ; procaine hydrochloride (Drug Houses of Australia) ; pentolinium tartrate (May & Baker) ; hexamethonium bromide (May & Baker) ; mecamylamine hydrochloride (Merck, Sharp & Dohme) ; tetrodotoxin (Sankyo).

Electrical stimuli were delivered through two straight silver electrodes placed vertically at opposite sides of the bath. Single stimuli of 0.2–0.5 msec duration were applied by a Grass stimulator. Voltages were used which produced maximal contractions.

The degree of potentiation or inhibition produced by varied Na^+ concentrations was estimated graphically from log-dose/response curves and expressed in terms of dose-ratio. Dose-response curves were obtained usually at three dose levels with 2–3 replicates of each dose. Since the dose-response curves in normal and altered conditions were not always parallel, estimates of dose-ratio were made towards each end of the linear segment of the dose-response curve.

RESULTS

1. *Effects of variations in Na^+ content of medium on response to angiotensin*

In the preliminary report of Blair-West & McKenzie (1966) an increase of Na^+ concentration by 30–40 m-equiv/l. was estimated to potentiate the contractile response to angiotensin to a degree equivalent to a three-fold increase of dose-level. The degree of potentiation has since been found to vary considerably between experiments ; in 21 preparations, responding in both normal and high Na^+ Krebs solution (plus 30–40

m-equiv/l.) to at least 3-dose levels of angiotensin, the mean dose ratio was 0.42. On the other hand, responses to angiotensin in low Na^+ Krebs solution (minus 30–40 m-equiv/l.) were depressed, the ratio of equivalent doses being 1.84 (Table 1).

TABLE 1

DISPLACEMENTS OF DOSE-RESPONSE RELATIONSHIPS (EXPRESSED AS DOSE-RATIO OF STIMULANT PRODUCING EQUIVALENT RESPONSE IN NORMAL KREBS SOLUTION) IN KREBS SOLUTION CONTAINING EXCESS OR DEFICIT OF SODIUM

Stimulant	Sodium level (m-equiv/l.)	Experiments (no.)	Mean dose-ratio	Standard error
Angiotensin II	180	21	0·42	0·016
	110	7	1·84	0·18
Acetylcholine	180	6	1·82	0·32
	110	5	1·86	0·23

The effects of these high and low Na^+ concentrations on the levels of response to angiotensin were not due to changes in the osmotic concentration of the bathing fluid. When the Na^+ concentration was reduced, the deficit of NaCl was always made up by the equivalent osmolar concentration of sucrose. The osmotic effect of the high Na^+ concentration was tested in control experiments where the osmolar concentration was increased to the same degree by addition of sucrose; equivalent osmotic increase with sucrose had no significant effect on the dose/response curve for angiotensin.

To a limited extent, the effect of variation in the Na^+ concentration increased with the magnitude of the variation. With stepwise increments of Na^+ concentration of 20 m-equiv/l. from 140 m-equiv/l. to 200 m-equiv/l. the dose response curves were moved to the left up to 180 m-equiv/l., but further increase to 200 m-equiv/l. produced variable effects. With successive decrements of 20 m-equiv/l., the total osmolar concentration being held constant with sucrose supplements, there was a fairly regular decline of responses to angiotensin down to a Na^+ concentration of 80 m-equiv/l. (Fig. 1).

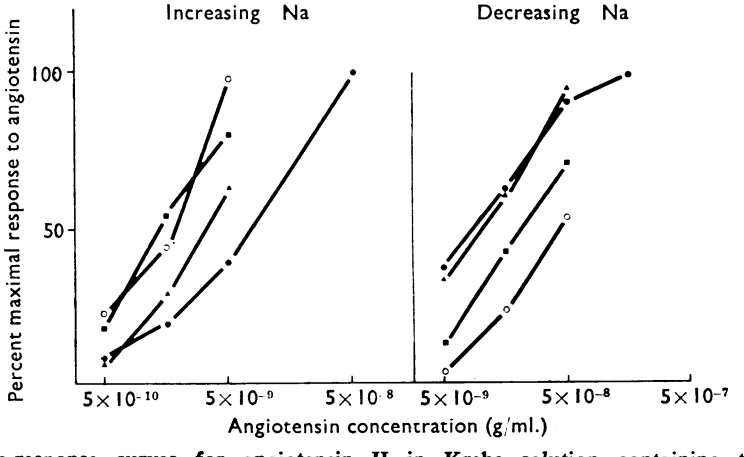

Fig. 1. Dose-response curves for angiotensin II in Krebs solution containing the following concentrations of Na^+: (a) left-hand side: 140 m-equiv/l. (●), 160 m-equiv/l. (▲), 180 m-equiv/l. (■), 200 m-equiv/l. (○); (b) right-hand side: 140 m-equiv/l. (●), 120 m-equiv/l. (▲), 100 m-equiv/l. (■), 80 m-equiv/l. (○).

The time-course of contractile responses to angiotensin depended on the dose added to the bath. At low doses the preparations shortened in small steps consisting of rhythmic contraction and incomplete relaxation. At intermediate doses the time-course of overall contraction was faster, so that a plateau was reached at which rhythmic contractions continued until wash-out. At maximal doses the response rose sharply to a peak, then fell to a plateau, upon which a series of high-amplitude rhythmic beats was often superimposed. In high Na^+ Krebs solution, both the initial peak and the plateau were increased in height (Fig. 2).

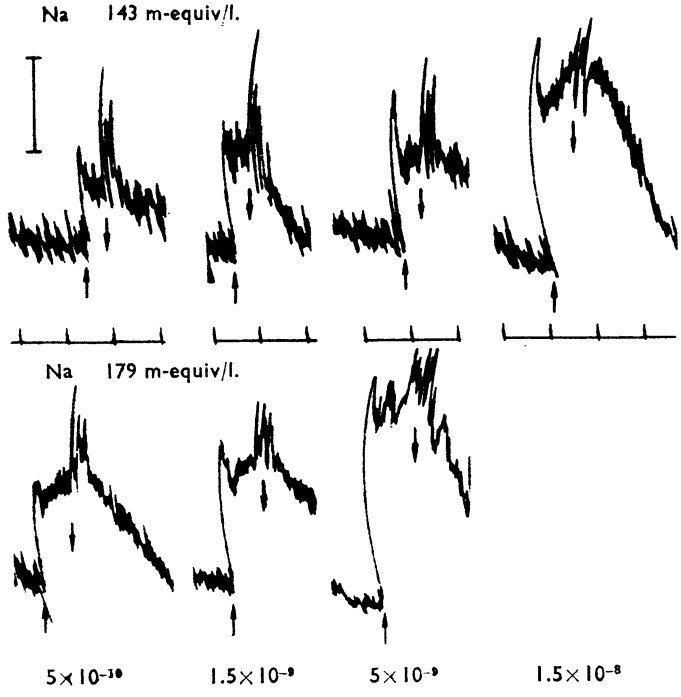

Fig. 2. The time-course of contractions of an isolated ileum preparation to angiotensin II at the stated concentrations in normal (143 m-equiv/l.) and in high sodium (179 m-equiv/l.) Krebs solution. Angiotensin added at (↑) and washed out at (↓). Time marker in min; vertical calibration bar 100 mg-tension.

Both high and low Na^+ concentrations depressed the responses to ACh, giving a dose-ratio of approximately two (Table 1). The effects of high Na^+ on responses to angiotensin and to DMPP were compared at approximately equiactive doses (5×10^{-9} g/ml. and 5×10^{-7} g/ml. respectively) in six preparations. An elevation of Na^+ concentration by 30–40 m-equiv/l. increased the response to angiotensin, with a fall towards or below the initial level on returning to normal Krebs solution. The response to DMPP was reduced by high Na^+ in three of these experiments and slightly increased in three experiments. In one of the latter group, the response increased still further on returning to normal Krebs solution (Fig. 3).

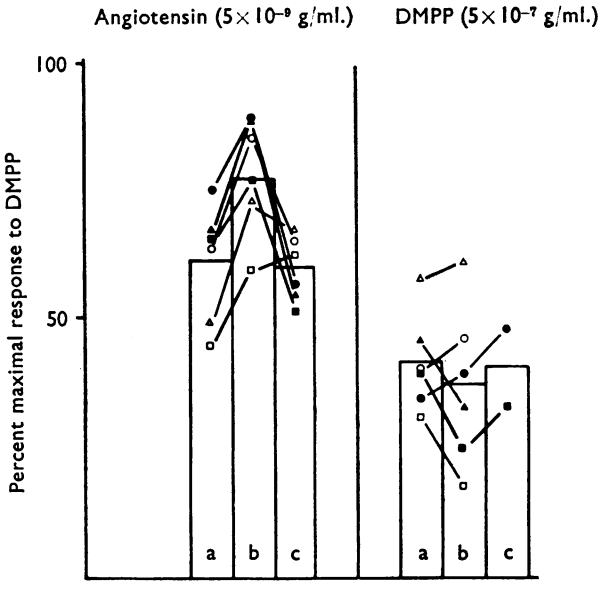

Fig. 3. Contractile responses of six isolated ileum preparations to angiotensin II (5×10^{-9} g/ml.) and DMPP (5×10^{-7} g/ml.) when the medium was changed in the sequence; (a) normal Krebs solution, (b) high sodium Krebs solution, and (c) normal Krebs solution. Each symbol denotes one preparation. Height of column indicates mean response of all preparations.

2. *Effects of blocking agents on contractile responses to angiotensin, DMPP, acetylcholine and electrical stimulation*

The effects of various types of pharmacological blockade on responses to each stimulating agent were expressed as percentage depression of response and where possible in terms of dose-ratio. The results are summarized in Table 2 and illustrated for representative single preparations in Figs. 4–7. In many instances of non-competitive blockade, the slope and the maximal response level of the log dose-response curves were depressed. Therefore a range of equivalent dose-ratios was estimated from measurements at each end of the plotted curves, and percentage depression measured at the geometric mean of the stimulant dose-range. No dose-ratio was estimated when insignificant or no responses were observed in the presence of an antagonist, over the range of stimulant doses used. In some experiments the effect of a blocking-agent was tested using single doses of each stimulant chosen to produce approximately equal magnitudes of response, in which case dose-ratios were indeterminate.

(*a*) *Ganglion-blocking agents* (Table 2)

Pentolinium (10^{-4} g/ml.) completely blocked the response to a maximally-effective dose (5×10^{-6} g/ml.) of DMPP, and at 10^{-5} g/ml. the dose-ratio was 50–100. With pentolinium (10^{-4} g/ml.), contractile responses to the four stimulating agents were reduced in the rank-order: DMPP>ACh>electrical stimulation>angiotensin. With pentolinium (10^{-5} g/ml.) the order of antagonism was: DMPP>ACh>angiotensin>electrical stimula-

TABLE 2

EFFECTS OF PHARMACOLOGICAL BLOCKING AGENTS ON CONTRACTILE RESPONSES OF GUINEA-PIG ILEUM TO ANGIOTENSIN II AND OTHER STIMULATING AGENTS

Blocking agent and concentration (g/ml.)		Effect on response to: Angiotensin Expts. (no.)	Angiotensin Mean % depression* (range)	Angiotensin Dose-ratio range†	Acetylcholine Expts. (no.)	Acetylcholine Mean % depression* (range)	Acetylcholine Dose-ratio range†	DMPP Expts. (no.)	DMPP Mean % depression* (range)	DMPP Dose-ratio range†	Electrical stimulation Expts. (no.)	Electrical stimulation Mean % depression* (range)
Pentolinium	10^{-4}	1	10	1·0–1·5	1	65	5·0	1	100	(No response)	1	25
	10^{-5}	3	37 (22–60)	1·5–5·0	3	67 (57–75)	5·0–10	3	91 (87–95)	50–100	2	25 (5–35)
	10^{-6}	1	8	1·0–1·5	1	60	3·3–5·0	1	20	2·0–3·3	1	8
Hexamethonium	10^{-5}	1	12	1·0–3·3	1	40	3·3–5·0	1	37	3·3–10	1	30
Mecamylamine	10^{-5}	1	25	2·0–2·5	1	74	3·3–5·0	1	100	(No response)		
	10^{-6}	2	15 (0–30)	0·7–2·5	1	78	2·5–10	2	97 (95–100)	>100	2	35 (34–36)
Tetrodotoxin	10^{-7}	12	67 (28–78)	3·3–20†	9	31 (6–59)	7·5–11	10	97 (90–100)	(Insignif. response)	6	100
	10^{-8}	2	40 (36–42)	Indeterminate	2	45 (38–48)	Indeterminate	2	72 (70–74)	Indeterminate	2	60 (42–77)
Hemicholinium	10^{-4}	2	71 (70–71)	10–100†	2	97	(Insignif. response)	2	100	(No response)	2	93 (92–94)
	10^{-5}	5	67 (59–73)	10–17	3	95	(Insignif. response)	5	97 (90–100)	(Insignif. response)	1	85
Atropine	10^{-4}	1	75	50–100†				1	100	(No response)		
	10^{-5}	4	51 (36–73)	5·0–33†				3	94 (89–100)	(Insignif.		
	10^{-6}	4	69 (62–76)	10–100†	4	100	10^{4}–10^{6}	4	92 (91–100)	response)	2	92 (88–95)
	10^{-8}	2	40 (39–41)	Indeterminate	2	100	Indeterminate	2	80 (76–88)	Indeterminate	2	88
	10^{-10}	1	27	Indeterminate	1	44	Indeterminate	1	28	Indeterminate	1	48

* Per cent depression of response at geometric mean of stimulant dose in each experiment.
† Ratio by which test dose of stimulant would be modified to match the response obtained in absence of blocking drug.
a Estimated in face of non-parallel log dose/response relationship (see text).

tion (Fig. 4) but with 10^{-6} g/ml. the response to ACh was the most severely reduced, followed by that to DMPP, with insignificant effects on responses to angiotensin and electrical stimulation.

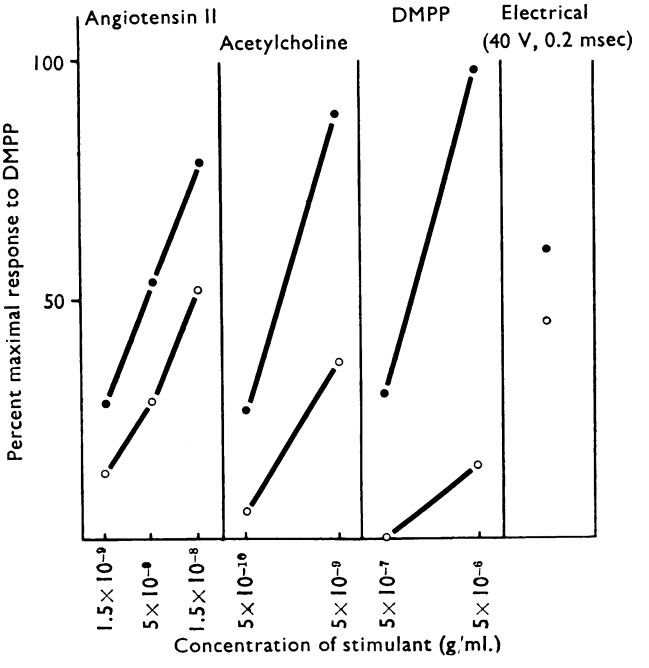

Fig. 4. Contractile responses of an isolated ileum preparation to angiotensin II acetylcholine, DMPP and electrical stimulation in normal Krebs solution (●) and in normal Krebs solution containing 10^{-5} g/ml. pentolinium tartrate (○).

Hexamethonium was used only at 10^{-5} g/ml., at which concentration the dose-ratio for DMPP was 3.3–10. The responses to ACh and electrical stimulation were reduced to approximately the same extent, while that to angiotensin was affected only slightly (dose-ratio 1.0–3.3).

Mecamylamine (10^{-6} g/ml.) almost completely blocked response to DMPP at a maximally-effective dose. At 10^{-6} to 10^{-5} g/ml. the effectiveness of mecamylamine against the stimulating agents was similar to that of pentolinium at 10^{-4} g/ml.

(*b*) *Nerve-blocking agents* (Table 2)

Procaine (10^{-4} g/ml.) had little detectable effect on the response to angiotensin, but severely depressed that to DMPP. There was evidence for direct effects of procaine on the smooth muscle, since the amplitude and occurrence of sporadic spontaneous contractions increased so as to obscure the patterns of antagonistic action.

Tetrodotoxin (10^{-8} g/ml.) depressed the agonist effects in the order DMPP>electrical stimulation>ACh, angiotensin. At 10^{-7} g/ml., the order of depression was: DMPP and

electrical stimulation>angiotensin>ACh (Fig. 5). At 10^{-7} g/ml., tetrodotoxin blocked the responses to DMPP and electrical stimulation either completely or nearly so. Its effectiveness against angiotensin and acetylcholine varied widely between preparations. Responses to angiotensin were more severely depressed than equivalent responses to ACh.

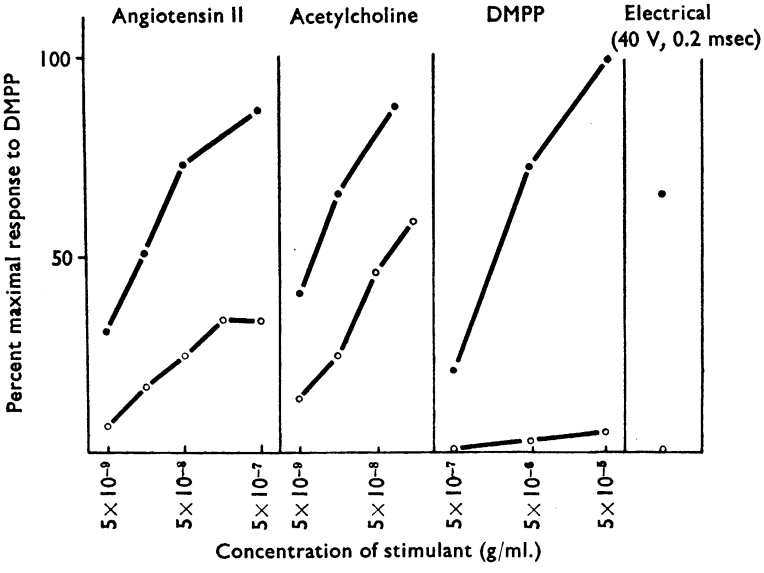

Fig. 5. Contractile responses of an isolated ileum preparation to angiotensin II, acetylcholine, DMPP and electrical stimulation in normal Krebs solution (●) and in normal Krebs solution containing 10^{-7} g/ml. tetrodotoxin (○).

Hemicholinium is included under nerve-blockers for convenience, although its major action is to reduce the availability of transmitter at cholinergic nerve terminals (Schueler, 1960). In the presence of hemicholinium (10^{-5} to 10^{-4} g/ml.) responses to repeated electrical stimulation were gradually reduced until after about 1 hr, the magnitude of contraction became steady at 5 to 15% of the original level. The responses to DMPP and ACh were then depressed almost to zero. With hemicholinium (10^{-5} g/ml.), the dose-ratio for angiotensin was 10–17 which was similar to the effect of tetrodotoxin at 10^{-7} g/ml. (Fig. 6).

(*c*) *Blockade of muscarinic receptors* (Table 2)

At concentrations of 10^{-8} g/ml. and above, atropine completely blocked responses to doses of acetylcholine which caused near-maximal contractions in atropine-free media. The responses to DMPP and electrical stimulation were reduced to insignificant levels, while those to angiotensin were depressed variably (dose-ratio 5.0–100). In the presence of atropine as concentrated as 10^{-4} g/ml., 25% of the response to angiotensin remained. With atropine (10^{-6} g/ml.) the response to acetylcholine could be restored only by a very large increase of dose; for restoration of a 50% response level, the dose-ratio was approximately 10,000 (Fig. 7). At this atropine concentration, the response to angiotensin

reached a maximum level at the same dose-range as before atropine was added—namely, 5×10^{-8} to 5×10^{-7} g/ml.—but this new maximal response reached only 40% of the original level.

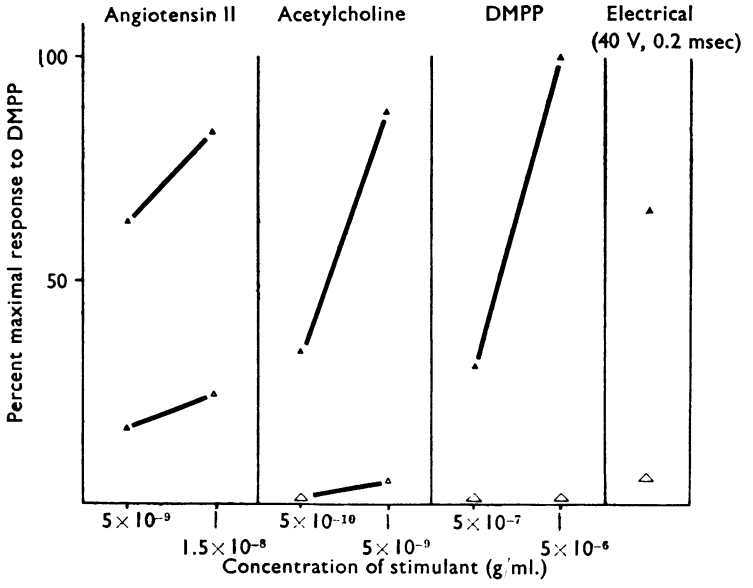

Fig. 6. Contractile responses of an isolated ileum preparation to angiotensin II, acetylcholine, DMPP and electrical stimulation in normal Krebs solution (▲) and in normal Krebs solution containing 10^{-4} g/ml. hemicholinium −3 (△).

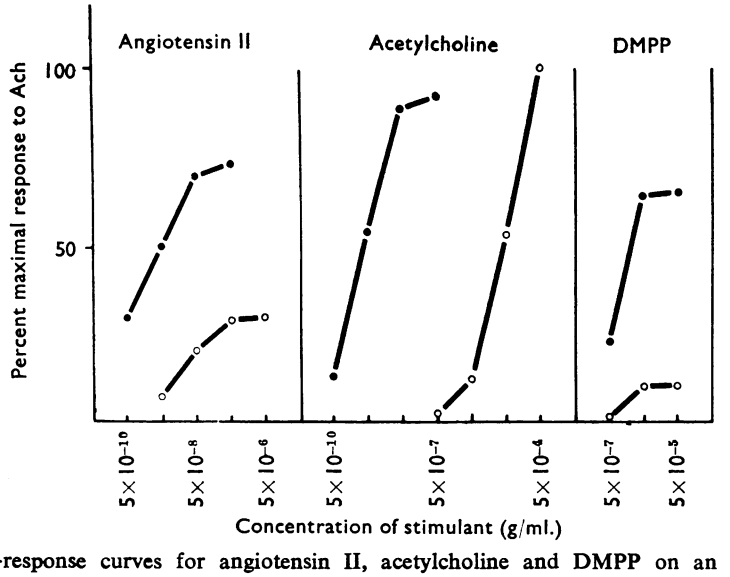

Fig. 7. Dose-response curves for angiotensin II, acetylcholine and DMPP on an isolated ileum preparation in normal Krebs solution (●), and in Krebs solution containing atropine sulphate at 10^{-6} g/ml. (○).

(d) Simultaneous blockade of axones and muscarinic receptors

In the presence of both atropine (10^{-6} to 10^{-5} g/ml.) and tetrodotoxin (10^{-7} g/ml.) the response to angiotensin was reduced (range of dose-ratio 3–30) to approximately the extent observed in the presence of either antagonist alone. Thus, in one experiment after atropine (10^{-5} g/ml.) had reduced the response with a dose-ratio range of 3–20 the addition of tetrodotoxin caused no further depression of response (Fig. 8).

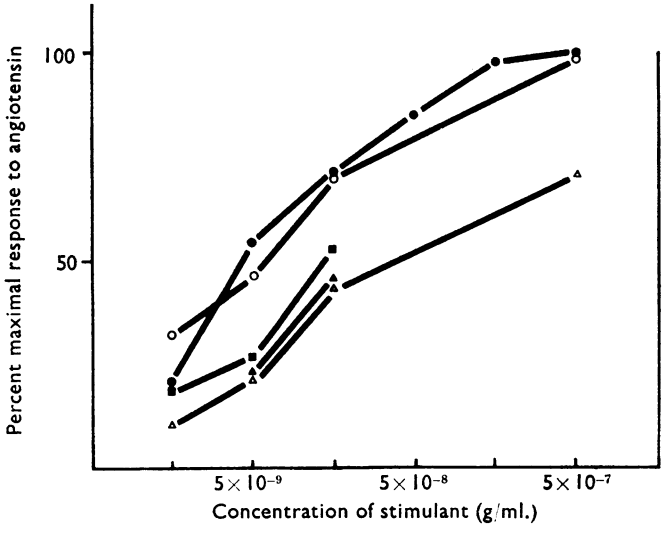

Fig. 8. Dose-response curves for angiotensin II on an isolated ileum preparation in: normal Krebs solution (●), normal Krebs solution containing 10^{-5} g/ml. atropine sulphate (▲), normal Krebs solution containing 10^{-5} g/ml. atropine sulphate and 10^{-7} g/ml. tetrodotoxin (■), high sodium Krebs solution containing 10^{-5} g/ml. atropine sulphate and 10^{-7} g/ml. tetrodotoxin (○), and normal Krebs solution containing 10^{-5} g/ml. atropine sulphate and 10^{-7} g/ml. tetrodoxtin (△), in that sequence.

3. *Influence of high Na^+concentration on contractile responses to angiotensin and other stimulants in the presence of blocking agents*

(a) Tetrodotoxin

In the presence of tetrodotoxin (10^{-7} g/ml.) an increase of Na^+ concentration of the bathing solution by 40 m-equiv/l. was still capable of potentiating the residual response to angiotensin. The influence of high Na^+ concentration was greatest in preparations in which the responses to angiotensin had been least depressed by tetrodotoxin, and in one experiment raised Na^+ concentration increased the responses to levels above those initially observed in the absence of the blocking agent (Fig. 9). The dose-ratio for equivalent effect was 0.3 compared with the initial curve in normal Krebs solution, and 0.1 compared with the curve in normal Krebs solution plus tetrodotoxin. In this experiment, tetrodotoxin had reduced the response with a dose-ratio of 3. In another experiment, the response was more severely reduced in tetrodotoxin (dose-ratio 10–30), and in this case the potentiatiing effect of the high Na^+ concentration was smaller. In each of these experiments, the response to DMPP (5×10^{-6} g/ml.) was almost abolished by tetrodotoxin. It was unchanged or further depressed by increasing the Na^+ concentration.

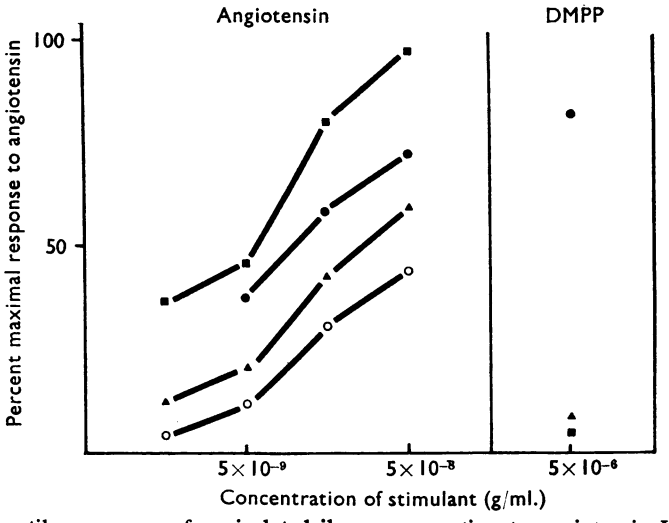

Fig. 9. The contractile responses of an isolated ileum preparation to angiotensin II and DMPP in: normal Krebs solution (●), normal Krebs solution containing 10^{-7} g/ml. tetrodotoxin (▲), high sodium Krebs solution containing 10^{-7} g/ml. tetrodotoxin (■), and normal Krebs solution containing 10^{-7} g/ml. tetrodotoxin (○) in that sequence.

(b) Atropine

In the presence of atropine (10^{-5} g/ml.), increasing the Na^+ concentration by 40 m-equiv/l. increased the response to angiotensin above the depressed level in normal Krebs solution containing atropine (Fig. 10). The potentiation was equivalent to a 0.3 to

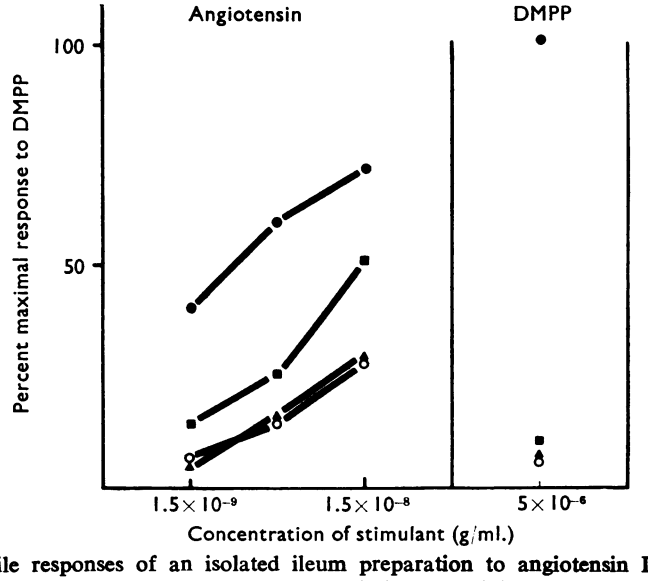

Fig. 10. Contractile responses of an isolated ileum preparation to angiotensin II and DMPP in: normal Krebs solution (●), in normal Krebs solution containing 10^{-5} g/ml. atropine sulphate (▲), high sodium Krebs solution containing 10^{-5} g/ml. atropine (■), and in normal Krebs solution containing 10^{-5} g/ml. atropine (○).

0.1 dose-ratio for angiotensin, and in one experiment restored the responses to the level observed before addition of atropine. The response to a maximally-effective dose (5×10^{-6} g/ml.) of DMPP was depressed by atropine to a small fraction of its original level and was then slightly increased by high Na^+in two experiments.

In one experiment, atropine at 10^{-4} g/ml. reduced the response to angiotensin with a dose-ratio of approximately 100. In the high Na^+ medium containing the same concentration of atropine the response to angiotensin was potentiated with a dose-ratio of 0.03–0.1.

(*c*) *Atropine and tetrodotoxin*

In the simultaneous presence of atropine (10^{-5} g/ml.) and tetrodotoxin (10^{-7} g/ml.) an increase of Na^+ concentration by 40 m-equiv/l. potentiated the residual responses to angiotensin with a 0.3 to 0.1 dose-ratio. In one experiment the potentiation was sufficient to restore the dose-response curve to the position occupied before addition of the blocking-agents (Fig. 8).

DISCUSSION

Khairallah, Vadaparampil & Page (1965) found that progressive reduction of the Na^+ concentration of Tyrode solution reduced and finally abolished responses of guinea-pig ileum to angiotensin, but found no effect on raising the Na^+ content from 140 m-equiv/l. to 240 m-equiv/l.

In the present study increase of external Na^+ concentration up to 200 m-equiv/l. potentiated the contractile response to angiotensin and reduction down to 80 m-equiv/l. reduced the response. The effects of high Na^+ solution on the contractile response of guinea-pig ileum to angiotensin and to ACh were opposite in direction, which eliminates the possibility that the potentiation of response to angiotensin merely reflects the potentiation of a response to liberated ACh. It also makes it unlikely that high Na^+ is acting on the contractile mechanism of the smooth muscle, irrespective of the initiating stimulus. Nor can the influence of high Na^+ be attributed to an osmotic action, for an equivalent osmotic loading with sucrose had no effect on the dose-response relationship for angiotensin. High Na^+ must influence the contractile response at a site of action of angiotensin itself, or on a mechanism mediating its effect. The problem is one of distinguishing between the smooth muscle cells and the excitatory intramural nervous elements as sites where the action of angiotensin may be potentiated.

By pharmacological blockade, individual steps in the excitatory pathway could be tested as sites of angiotensin potentiation, provided the blocking-agents could be shown to exert selective action in the experimental conditions used. When ganglionic nicotinic receptors were completely blocked, as judged by responses to DMPP, there was a minor depression of the response to angiotensin, suggesting that the latter had no significant preganglionic stimulant action. On the other hand, responses to ACh were severely depressed by ganglionic blockers, an effect not commonly observed with isotonic recording methods. Tetrodotoxin also reduced responses to ACh by about 50%. Isometric or auxotonic recording methods more readily detect failure of a fraction of the elements contributing in parallel to a contractile response (Wilkie, 1962), and in the present

experiments revealed a significant indirect contribution to the effect of exogenous ACh, probably by stimulation of ganglion cells.

Tetrodotoxin (10^{-7} g/ml.), hemicholinium (10^{-5} g/ml.) and atropine (10^{-6} g/ml.) each reduced the responses to angiotensin incompletely and to approximately the same extent, but the latter two drugs depressed responses to ACh completely or nearly so. The principal action of hemicholinium is considered to be depletion of transmitter availability at cholinergic nerve terminals (Long, 1961), but it can also block post-synaptic actions of ACh as shown by Martin & Oorkand (1961). Combination of these effects would strongly inhibit the direct and indirect actions of added ACh, the direct fraction of response to angiotensin remaining unaffected by either hemicholinium or atropine. Evidence reviewed by Kao (1966) demonstrates that tetrodotoxin blocks propagated action potentials in nerve or striated muscle fibres but has no significant action on responses of smooth muscle cells to direct stimuli, a conclusion reinforced for guinea-pig taemia coli by Kuriyama, Osa & Toida (1966) and for guinea-pig ileum by Ogura, Mori & Watanabe (1966). Gershon (1966) has recommended tetrodotoxin at 10^{-7} g/ml. for functional denervation of smooth muscle preparations. Atropine, at concentrations 100 times those required to block just-maximal response to ACh, does not eliminate a residual response of guinea-pig ileum to angiotensin (Blair-West & McKenzie, 1966). The fraction of the response to angiotensin eliminated by these blocking-agents must be due to stimulation of excitatory ganglia or their terminals. The residual contraction is probably a direct response of the smooth muscle.

Thus the action of angiotensin might be potentiated in high Na^+ solutions by any of three mechanisms: (1) potentiation of its stimulant action on intramural excitatory ganglia; (2) potentiation of ACh release at excitatory post-ganglionic terminals; (3) potentiation of the direct response of smooth muscle. Either tetrodotoxin or atropine appeared to be suitably selective agents for eliminating the effect of ganglionic stimulation, while only atropine would counteract that of possible stimulation of post-ganglionic terminals.

High Na^+ concentration failed to potentiate responses to DMPP, and so appeared not to increase the general excitability of intramural ganglia. Any ganglionic site of potentiation would thus involve specifically a non-nicotinic receptor stimulated by angiotensin (Trendelenburg, 1966). The failure of axonal blockade by tetrodotoxin to abolish response potentiation would seem to eliminate any ganglionic site. However, if some post-ganglionic axones were resistant to tetrodotoxin, transmitter release at the terminals might be increased sufficiently to potentiate the contractile response. This increase would need to be such as to overcome atropine blockade because the residual response was potentiated in the presence, with tetrodotoxin, of atropine at a dose-level ten times that capable of blocking the complete range of responses to ACh in the unblocked preparation. A 10,000-fold increase in the concentration of ACh was required to overcome blockade by atropine at 10^{-6} g/ml.

In the absence of blocking agents, the average potentiation of response to angiotensin was equivalent to increasing the dose-level in normal Na^+ by a factor of 2.36. Since the log dose-response lines for angiotensin and ACh were approximately parallel, increasing the angiotensin concentration by this factor would have the same effect as a similar multiplication of ACh concentration. But in high Na^+ solutions the response to ACh

was depressed, to the equivalent of a 2:1 dose-ratio. Thus, to achieve the average degree of response potentiation for angiotensin, the amount of ACh liberated as a result of ganglionic potentiation in high Na^+ would be increased by a factor not greater than 5. This increase in transmitter release would be inadequate to account for the effect of high Na^+ on the residual response in the presence of atropine (which would require a factor of 10,000) or in the simultaneous presence of atropine and tetrodotoxin. The effect of high Na^+ on the contractile response cannot, then, be attributed to potentiation of a ganglionic response to angiotensin.

By the same argument, high Na^+ concentration does not potentiate the release of ACh from post-ganglionic terminals. Furthermore, in the absence of blocking agents, there was no significant potentiation of responses to DMPP, although the small residual response in the presence of atropine was increased slightly by high Na^+ in two experiments. But in the presence of tetrodotoxin, when residual responses remained to DMPP or electrical stimulation, there was no sign of their potentiation by high Na^+. If such residual responses signify persisting function in tetrodotoxin-resistant axones, their failure to be potentiated argues further against an increased ACh release from post-ganglionic terminals.

Other than the direct action of angiotensin on smooth muscle cells, the only possible sites of potentiation remaining involve non-cholinergic mediators of the contractile response. Addition of tetrodotoxin did not reduce any further the responses to angiotensin obtained in the presence of atropine, which makes it unlikely that non-cholinergic excitatory nerves contribute to them. It is possible that angiotensin stimulates the release of known smooth muscle excitants from local stores, but Walaszek, Huggins & Smith (1963) have reported that anti-histaminics and serotonin antagonists have no effect on the response of guinea-pig ileum to angiotensin. The contractile response to angiotensin may be mediated, in part, by as yet unrecognized local excitants. Until such excitants are established, the evidence suggests that an increase in Na^+ concentration of the medium, by about 40 m-equiv/l., potentiates a direct unique action of angiotensin on intestinal smooth muscle.

SUMMARY

1. The contractile response of isolated guinea-pig ileum to angiotensin II was increased in high sodium media and reduced in low sodium media.

2. The potentiation of the response to angiotensin was not abolished by blockade of the neurally-mediated fraction of the response by tetrodotoxin or atropine.

3. It was not attributable to an increased response of the intramural ganglia, to increase acetylcholine release or to an effect on the mechanisms of contraction subsequent to excitation.

4. It was concluded that increased sodium concentration influences the direct action of angiotensin on smooth muscle cells.

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