The results suggest the existence of receptors of different structures. It is not yet possible to say if there are three different types of receptors, each one particular for rat colon, rat uterus and rabbit aorta, or if instead there are two types, which have variable concentrations in the different organs tested.

Angiotensin auto-potentiation

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It has been reported that the response to angiotensin is influenced by a preceding administration of the peptide. On whole animals as well as on isolated preparations, tachyphylaxis occurred after repeated administration (Khairallah, Page, Bumpus & Türker, 1966; Godfraind, 1968a). However, in the longitudinal smooth muscle of the guinea-pig ileum, either auto-potentiation or tachyphylaxis occurred according to the rate of dissociation from the receptor (Godfraind, 1968b).

The present experiments were designed to determine whether the potentiation was due to a specific modification of the angiotensin receptor or to a non-specific change in tissue responsiveness. Helical strips of guinea-pig aorta, bathed in Krebs solution, were contracted by cumulative increments of angiotensin dosage (from 10^{-10} to 10^{-6}M). When the period at rest between two successive treatments was 90 min, the maximum response was increased to 153% (mean of ten experiments); however, the ED50 was in both successive series equal to 10^{-8}M.

In other experiments performed on the longitudinal smooth muscle of the guinea-pig ileum, the maximum response of the preparation stimulated by acetylcholine was increased by the presence of angiotensin (10^{-8}M). Furthermore, the aspecific desensitization evoked by acetylcholine, according to Paton & Rothschild (1965), was reduced when the desensitizing dose was added in the presence of angiotensin. This action was associated with a reduction of tissue ionic changes due to acetylcholine.

Longitudinal smooth muscles were immersed in a phosphate-free solution at 37° C for 2 h after dissection. The composition (mM) of this solution was as follows: NaCl 122, $NaHCO_3$ 15, KCl 5·9, $CaCl_2$ 2·5, $MgCl_2$ 1·25 and glucose 11·5. Thereafter, the muscles were transferred for 5 min in the same solution containing acetylcholine (100 μg/ml); the Na, K and Ca content were measured and compared with that of controls. A loss of 12·3 mmoles K/kg wet weight was compensated by a gain of 12·8 mmoles Na/kg ($n=10$), the Ca remaining constant. When acetylcholine was added to the solution containing angiotensin (10^{-5}M), ionic changes due to acetylcholine were reduced: the gain of Na was 4·5 mmoles/kg and the loss of K was 4·5 mmoles/kg ($n=10$). Na, K and Ca content of muscles treated with angiotensin (10^{-5}M) were not modified as compared with controls.

In another series of experiments, after 2 h in the phosphate free solution as above, the muscles were incubated for 1 h in the same solution but containing only 0·5 mM $CaCl_2$.

The reduction of the calcium concentration of the incubating fluid altered the ionic content, there was a net gain in Na (from $101{\cdot}7\pm0{\cdot}8$ mmoles/kg; $n=15$ to $114{\cdot}4\pm0{\cdot}8$ mmoles/kg; $n=15$), and a net loss of Ca (from $4{\cdot}6\pm0{\cdot}1$ mmoles/kg; $n=15$ to $2\pm$

0·1 mmoles/kg; $n=15$). The addition of acetylcholine (100 μg/ml) for 5 min induced a further increase of the sodium content (122·2$\pm$0·9 mmoles/kg; $n=13$) and a further decrease of calcium (1·7$\pm$0·1 mmoles/kg; $n=13$). In the presence of angiotensin (10^{-7}M) these ionic changes were reduced.

These observations show that angiotensin induced a net sodium efflux in smooth muscle desensitized by acetylcholine; when the calcium content of the medium was reduced this sodium efflux was associated with a net calcium influx. This could be due to an activation of a coupling between an inward movement of calcium and an outward movement of sodium. Such an activation could explain the increase in tissue responsiveness in the various experimental conditions here described.

REFERENCES

GODFRAIND, T. (1968a). Pharmacology of angiotensin antagonists. In *Pharmacology of Hormonal Polypeptides and Proteins*, pp. 497–506. New York: Plenum Press.

GODFRAIND, T. (1968b). Réponse contractile de muscles lisses isolés à des stimulations répétées par l'angiotensine. *Archs int. Pharmacodyn. Thér.*, **176**, 464–467.

KHAIRALLAH, P. A., PAGE, I. H., BUMPUS, F. H. & TÜRKER, R. K. (1966). Angiotensin tachyphylaxis and its reversal. *Circulation Res.*, **19**, 247–254.

PATON, W. D. M. & ROTHSCHILD, A. M. (1965). The changes in response and in ionic content of smooth muscle produced by acetylcholine action and by calcium deficiency. *Brit. J. Pharmac. Chemother.*, **24**, 437–448.

Blockade of the effects of Valyl-5-angiotensinamide II, oxytocin, noradrenaline, 5-hydroxytryptamine and acetylcholine by guancydine

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Guancydine is a 1-cyano-3-*tert*-amylguanidine whose vasodepressor effects were attributed to a specific angiotensin blocking activity (Cummings, Welter, Grace & Lipchuck, 1968).

The contractile responses of the isolated uterus and colon from the rat were tested using a low Ca (0·6 mM) Krebs solution in order to avoid spontaneous contractions. Strips of rabbit aorta were suspended in Krebs solution with normal Ca (1·3 mM). The agonists used were Valyl-5-angiotensinamide II on colon, uterus and aorta; oxytocin on uterus; 5-hydroxytryptamine on uterus; acetylcholine on colon and noradrenaline on aorta. Guancydine, when used in concentrations higher than 10^{-4}M, antagonized in a non-competitive fashion the response to all of the agonists tested.

In another series of experiments, rat uterus and rat colon were suspended in a Krebs solution containing 2·5 mM Ca; the organs developed a strong rhythmic activity. Guancydine in concentrations higher than 10^{-7}M inhibited the spontaneous contractions. In higher concentrations (5×10^{-4}M) guancydine inhibited the action of oxytocin on net water influx of frogs (*Rana esculenta*) bladder epithelium.

In conclusion, guancydine inhibits smooth muscle contractility by a mechanism probably affecting the excitation-contraction coupling and this is the reason for the non-specific, non-competitive antagonism produced by guancydine on the actions of Valyl-5-angiotensinamide II, oxytocin, 5-hydroxytryptamine, acetylcholine and noradrenaline.

REFERENCE

CUMMINGS, J. R., WELTER, A. N., GRACE, J. L. & LIPCHUCK, L. M. (1968). Cardiovascular actions of guancydine in normotensive and hypertensive animals. *J. Pharmac. exp. Ther.*, **161**, 88–97.