Sodium Concentration and the Effect of Angiotensin II on Ileal Smooth Muscle

Angiotensin II is a potent proximate stimulus to aldosterone secretion[1], and the level of secretory response is inversely related to the plasma sodium concentration[2]. The pressor action of angiotensin is an increasing function of the total body sodium content[3-5]. These examples suggest that the ambient sodium concentration may modify actions of angiotensin on effector cells generally, but that the direction of the modification, in terms of measured response, may differ in various tissues. The present paper reports effects of changes in sodium concentration of the medium on the contractile response of isolated guinea-pig ileum to angiotensin.

Experiments were performed on 1–2 cm lengths of guinea-pig ileum suspended in Krebs bicarbonate Ringer bubbled with 5% CO_2 in O_2[6] at 37 °C in a 20 ml organ bath. Contractions were recorded with a Grass force-displacement transducer coupled to an Offner recorder. The bath was emptied and washed as soon as a response had passed its maximal value. In most experiments sodium concentration of the medium was varied by ± 30 meq./l. The tonicity of the low sodium solution was corrected by adding 60 mOsm./l of sucrose and the effect of the hypertonic sodium solution was compared with the effect of Ringer brought to equivalent tonicity with sucrose. Dose-response curves in standard Ringer were obtained for 0.1 ml additions of valine-5-angiotensin II amide (Ciba) or acetylcholine chloride (Roche). The log dose-response relationships were linear over the range $5 \cdot 10^{-10}$ to $5 \cdot 10^{-8}$ g/ml. Three-point dose-response curves were determined with randomized doses of either angiotensin or acetylcholine given at 4 min intervals for approximately 1 h periods in standard Ringer, then in high or low sodium Ringer, and then again in standard Ringer.

Figure 1 shows the results for angiotensin added to high and low sodium Ringer at two dose levels. The results are expressed as the mean deviation from the mean contractile response in standard Ringer. The mean deviations are plotted for responses grouped in the intervals 0–20 min and 20–60 min in high or low sodium Ringer and 0–20 min and 20–60 min after standard Ringer was replaced.

Increase of 30 meq./l NaCl in the medium increased the potency of angiotensin, often threefold. The potentiation occurred within 20 min and persisted for 60 min in all but one case. Replacement of standard Ringer always resulted in a large decline of response to angiotensin. The response to angiotensin was not increased in Ringer made 60 mOsm./l hypertonic by addition of sucrose. Reduction of the sodium content of the medium by 30 meq./l always caused a fall of the contractile response to added angiotensin. Usually the responsiveness to angiotensin increased when the standard medium was replaced but rarely recovered to control levels within 1 h. There was a small regular decline of response to control doses of angiotensin given every 4 min for a period of 2–3 h. When the sodium concentration of the Ringer was altered by ± 50 meq./l similar but larger alterations in contractile response to angiotensin were observed. The findings indicate that the reactivity to angiotensin of ileal smooth muscle is directly related to the sodium content of the medium.

The relative effect of atropine on the action of submaximal doses of angiotensin and ACh was examined (Figure 2). At $5 \cdot 10^{-8}$ g/ml atropine, the ACh effect was abolished and the angiotensin effect was reduced to about 40% of control level. Doses of angiotensin, ACh and the ganglion stimulant dimethylphenylpiperazinium iodide (DMPP, Fluka) were chosen to produce contractions of approximately equal peak size. Atropine at 10^{-6} g/ml reduced the response to angiotensin by 50–70%, but responses to ACh and DMPP were virtually abolished. The results are consistent with those of KHAIRALLAH and PAGE[7] in that they suggest that part of the action of angiotensin II on ileal muscle is mediated by ACh and a substantial fraction is not.

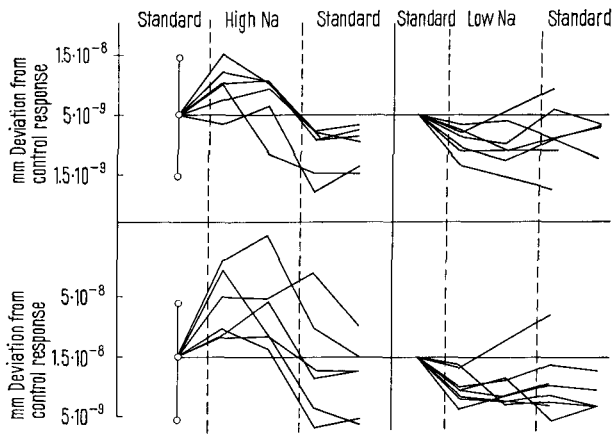

Fig. 1. Contractile response to angiotensin II.

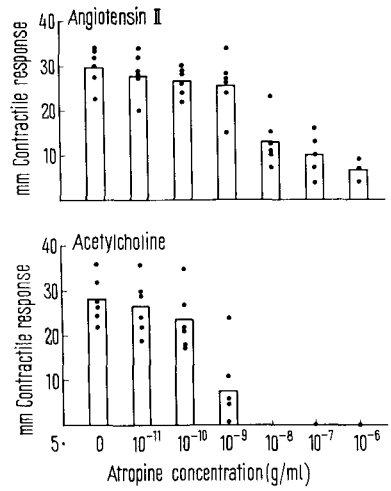

Fig. 2. Relative effect of atropine on the action of submaximal doses of angiotensin and ACh.

1 J. R. BLAIR-WEST, J. P. COGHLAN, D. A. DENTON, J. R. GODING, J. A. MUNRO, R. E. PETERSON, and MARELYN WINTOUR, J. clin. Invest. *41*, 1606 (1962).

2 J. R. BLAIR-WEST, J. P. COGHLAN, D. A. DENTON, J. R. GODING, MARELYN WINTOUR, and R. D. WRIGHT, Circ. Res. *17*, 386 (1965).

3 J. O. DAVIS, Circulation *25*, 1002 (1962).

4 J. R. BLAIR-WEST, J. P. COGHLAN, D. A. DENTON, J. R. GODING, J. A. MUNRO, and R. D. WRIGHT, Aust. J. expl Biol. med. Sci. *41*, 369 (1963).

5 J. H. LARAGH, P. J. CANNON, and R. P. AMES, Can. med. Ass. J. *90*, 248 (1964).

6 W. W. UMBREIT, R. H. BURRIS, and J. F. STAUFER, *Manometric Techniques* (Burgess Publ. Co., Minneapolis 1959), p. 149.

7 P. A. KHAIRALLAH and I. H. PAGE, Am. J. Physiol. *200*, 51 (1961).

This dual action of angiotensin II suggests several possibilities for the site of action of sodium concentration change and one possibility was that it might modify the action of liberated ACh. However, the high sodium medium did not augment the contractile response to added ACh, but in both high and low sodium media the response was reduced, often substantially (Figure 3). A small reduction of response to ACh occurred in standard Ringer over 2–3 h.

In view of this finding we examined the effect of raising the sodium concentration (+ 30 meq./l) in media containing atropine at 10^{-6} g/ml, which abolished the response to $5 \cdot 10^{-7}$ g/ml ACh. The potency of angiotensin II was increased approximately tenfold in the high sodium as compared with the normal sodium media.

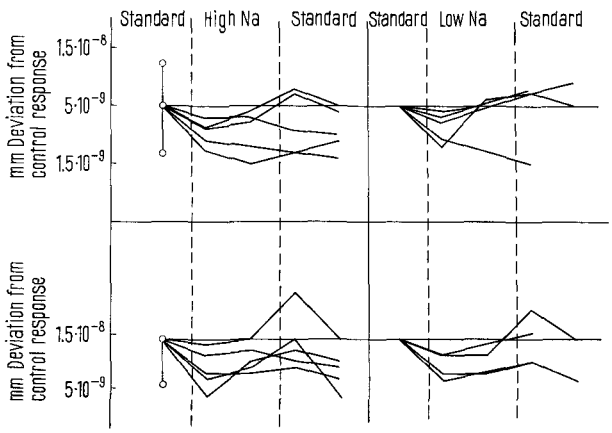

Fig. 3. Contractile response to acetylcholine.

KHAIRALLAH and PAGE[7] concluded that, besides the smooth muscle cells of the ileum, only the intramural post-ganglionic neurones respond directly to angiotensin. LEWIS and REIT[8] have demonstrated that angiotensin is indeed a potent stimulant of autonomic ganglia. There is the further possibility that angiotensin may release other mediators of the contractile response. Experiments are continuing to examine the possible sites where sodium may influence the action of angiotensin.

Zusammenfassung. Die kontraktile Reaktion des isolierten Meerschweinchen-Ileums auf Angiotensin II variierte direkt mit der Natriumkonzentration der Nährflüssigkeit, nicht aber bei ACh; Atropin (10^{-6} g/ml) reduzierte die Wirkung auf Angiotensin um 60–70% und hob die Wirkung auf äquivalente Mengen von ACh auf. In atropinhaltiger Ringerlösung potenzierte ein hoher Natriumgehalt (+ 30 meq./l) die Reaktion auf Angiotensin zehnfach.

J. R. BLAIR-WEST and J. S. MCKENZIE

Howard Florey Laboratories of Experimental Physiology, Department of Physiology, University of Melbourne, Parkville, N.2, Victoria (Australia),
January 14, 1966.

[8] G. P. LEWIS and E. REIT, J. Physiol. London *179*, 538 (1965).

Naringenin Inhibition on Explants in vitro of *Cichorium intybus* (Chicory)

Naringenin (5, 7, 4′-trihydroxyflavanone) is considered[1,2] one of the inhibitors regulating the dormancy of peach flower buds, while DENNIS and EDGERTON[3] and we ourselves[4] have shown that the naringenin level is not correlated to bud development. Besides, this substance considerably inhibits cell elongation[3,5] and in vitro causes a weak inhibition of proliferation, induced by 3-indoleacetic acid, of *Helianthus tuberosus* explants[6].

There are no data on naringenin inhibition of organogenesis in vitro. As chicory roots (*Cichorium intybus*) in vitro may form buds[7], we have studied naringenin's effect on this organogenesis.

Prismatic (1 cm) explants from sterile roots, containing vascular parenchyma, cambium and phloem, were utilized. These were placed in vitro with radical parts in a nutritive medium[8] with glucose 5% and agar 1.2%. Concentrations of naringenin (Calbiochem) between 10^{-4} and 10^{-7} *M* were utilized with a control in basal medium alone. 16 replications were utilized for every concentration. The cultures were randomized and grown in a culture room at 25 °C in continuous light of 1800 lux. The experiment was repeated thrice at different times with similar results. In the Table the results refer to a single experiment.

It is evident that naringenin 10^{-4} *M* completely blocks bud formation. Concentrations of naringenin between 10^{-5}

Naringenin effect on bud formation in explants in vitro of chicory roots: % of buds compared with control = 100

Molar concentrations of naringenin	Age of cultures (days) 7	9	11	13	15
0	100	236.61	245.70	250.86	256.02
10^{-4}	0.00	0.00	0.00	0.00	0.00
10^{-5}	10.81	106.39	150.12	177.39	188.21
10^{-6}	26.29	147.48	173.71	190.42	200.49
10^{-7}	49.14	147.42	157.25	188.21	196.56

[1] K. L. J. BLOMMAERT, Sci. Bull. Dep. Agric. For. Un. S.A. *368*, I (1955).
[2] C. H. HENDERSHOTT and D. R. WALKER, Proc. Am. Soc. hort. Sci. *74*, 121 (1959).
[3] F. G. JR. DENNIS and L. J. EDGERTON, Proc. Am. Soc. hort. Sci. *77*, 107 (1961).
[4] N. BAGNI, G. CRISTOFERI, and D. SERAFINI FRACASSINI, Atti del Congresso del Pesco, Verona 20–24 luglio 1965, in press.
[5] J. P. NITSCH and C. NITSCH, Bull. Soc. bot. Fr. *108*, 349 (1961).
[6] J. P. NITSCH and C. NITSCH, Bull. Soc. bot. Fr. *107*, 326 (1960).
[7] R. J. GAUTHERET, *La culture des tissus végétaux* (Masson et Cie, Paris 1959), p. 266.
[8] F. BERTOSSI, N. G. Bot. Ital. n. s. *66*, 497 (1959).