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EFFECT OF ANGIOTENSIN II ON ACTIVE TRANSPORT OF SODIUM BY TOAD BLADDER AND SKIN

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Abstract—Large amounts of angiotensin II (Hypertensine CIBA) were added repeatedly to the incubation fluid on the inner side of the isolated bladder and skin of *Bufo marinus*, without statistically significant inhibition of short-circuit current being demonstrable. Membranes exposed to aldosterone did not seem to be more responsive to angiotensin than untreated membranes. Rapid, extensive inactivation of angiotensin II, as judged from measurements of vasopressor activity, was a constant feature and had to be taken into account for proper experimental setting. The increase of short-circuit current due to vasopressin almost doubled in amplitude, but not in duration, when the toad bladder was exposed to angiotensin.

NUMEROUS clinical and experimental studies have been devoted to the effects of angiotensin II on the renal handling of sodium. In some animal species, this octapeptide brings about an increased sodium excretion. Thus injection of renal extracts containing renin was shown twenty five years ago to be followed rapidly, in rabbits, by a diuresis and a natriuresis.[1] As there was no significant change in creatinine clearance this effect was ascribed to inhibition of tubular reabsorption of water and sodium (chloride). The same was observed in rats;[2] in this species the phenomenon was abolished after adrenalectomy, and enhanced by aldosterone and ocytocin.[3] It should be remembered that angiotensin II is formed in the plasma after sequential action of renin and a "converting enzyme" on angiotensinogen, an alpha 2 globulin.[4, 5]

As to the diuretic effect of angiotensin II, in the rat it would be about four orders of magnitude greater than that of hydrochlorothiazide.[6] From studies in patients with cirrhosis, Laragh *et al.*[7] concluded that angiotensin II depressed tubular sodium reabsorption proximally and distally. Hypertensive subjects also respond to angiotensin II with a natriuresis.[8, 9]

In normal subjects on the other hand, sodium is retained by the kidney upon infusions with angiotensin II. The stimulation of aldosterone secretion ascribed to this peptide[10, 11] cannot account for the sodium retention observed were it only because the latter occurs in man in the absence of the suprarenal cortex.[13, 14]

Since the effect of angiotensin II on the normal kidney has a vascular component, involving a decrease in glomerular filtration rate and renal plasma flow, a dual mechanism of action of angiotensin II could be responsible for the apparent diversity of the data referred to. Prevalence of the renal heamodynamics changes over the decrease in tubular reabsorption of the filtered sodium would conceivably lead to sodium retention; excretion of sodium on the other hand could result from the reverse.

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The relative inaccuracy of our techniques for measurement of glomerular filtration rate renders adequate dissociation of both factors difficult. Furthermore, direct evaluation of a possible effect of angiotensin on sodium tranport by tubular cells is still lacking.

Because the urinary bladder and ventral skin of Amphibia are in many respects functionally similar to the distal portion of the mammalian kidney tubule, a study was undertaken of the action of angiotensin II on sodium transport by these membranes *in vitro*.

MATERIALS AND METHODS

Toads, *Bufo marinus*, were maintained either in shallow tap water or in moist peat before use. The ventral skin or the urinary bladder was dissected from pithed animals and symmetrical parts were mounted as diaphragms between pairs of conical Lucite chambers (internal diameter: 20 mm) both surfaces of each membrane being immersed in aerated frog Ringer's solution (in m equiv./l: Na, 117·5; K,2·0; Ca,2·0; Cl,119·0; HCO_3,2·5). Short-circuit current as well as transmembrane potential were measured at room temperature at 15-min intervals at least, according to the method of Ussing and Zerahn.[15]

Angiotensin II, synthetic val-5-angiotensin II amide (Hypertensin CIBA) was dissolved in Ringer's fluid shortly before being introduced on the serosal side in amounts and at intervals to be detailed. After variable periods of time samples were removed from the incubation solution for assay of angiotensin vasopressor activity. When used, *d*-Aldosterone (Aldocortene CIBA) was added to Ringer's fluid on the serosal side to achieve a steroid hormone concentration of 10^{-5}M. In several instances, before completion of the experiment antidiuretic hormone (Pitressin, Parke, Davis and Co) was introduced in fluid bathing the serosal surface at a final concentration of 50–100 mU/ml.

Inactivation of Angiotensin II was evaluated in 2 ways. The method of Fasciolo and Taquini[16] consists in the perfusion with Ringer's of the isolated hindlegs of the toad at a constant hydrostatic pressure through the abdominal aorta of the animal. The venous efflux was measured before and after addition of the material to be tested which was administered in 0·05 ml Ringer's. Assay was also carried out in the anaesthetized, vagotomized rat treated with pentapyrrolidinium tartrate.[17, 18] Blood pressure was measured directly from the carotid artery with a strain gauge. All samples were assayed by interpolation between two standards doses.

RESULTS

Addition of a single dose of angiotensin II to incubating toad bladders and skin so as to achieve a final concentration of 10^{-6} or 10^{-5} M failed to bring out rapid changes in sodium current. This prompted a study of inactivation of the peptide by toad bladder. It was found that angiotensin was completely inactivated after three hours of exposure to this membrane. From Table 1 it is obvious that with the incubated material, vasoconstriction could no longer be demonstrated, contrary to what resulted from injection of unincubated angiotensin. Angiotensin II (10^{-5} M) was assayed by the same method and by the rat assay after exposure to the inner surface of toad skin; more than 95 per cent of the vasoconstrictive effect was lost after one hour of incubation already. The tissue is largely responsible for this loss since only one fourth of the

TABLE 1. BIOLOGICAL INACTIVATION OF ANGIOTENSIN II BY THE TOAD BLADDER (METHOD OF FASCIOLO AND TAQUINI)

Experiment No.	Effect of fresh solution* (10^{-6} M)	Effect of incubated solution* (10^{-6} M)
1	38–39%	100%
5	28–87%	112%
7	0–47%	97%
8	1–71%	91%
		(10^{-5} M)
3	51–59%	97%
9	37–64%	96%

* The dripping rate after injection of the material to be tested is expressed relative to the baseline rate: the higher the value, the smaller the vasoconstrictive effect. Incubated samples were assayed between two standard samples, because of interference on account of tachyphylaxis.

pressor activity disappeared in 1 hr when angiotensin was added to the chambers after removal of the tissue.

Because of the lack of immediate effect on sodium transport and in view of this extensive inactivation large amounts of angiotensin II (70 μg) were added in twelve instances to the incubation medium (7 ml) on the serosal side of the toad bladder every 15 min for 150 min. Only one membrane of each pair was treated as described, the matched bladder half serving as a reference. The results were analysed according to the method described previously[19] i.e. the spontaneous change in activity of the untreated membrane within a set period of time was subtracted from the corresponding activity change of the paired, treated preparation. As seen in Table 2, after two hours and a half of incubation, the short-circuit current of the bladders exposed to angiotensin

TABLE 2. INFLUENCE OF ANGIOTENSIN II ON TRANSMEMBRANE POTENTIAL AND SHORT-CIRCUIT CURRENT OF THE ISOLATED TOAD BLADDER (MEANS OF 12 PAIRED EXPERIMENTS).

Short-circuit current (μA)			Transmembrane potential (mV)	
Untreated membrane	Matched membrane exposed to angiotensin II*	Time (min)	Untreated membrane	Matched membrane exposed to angiotensin II*
143·5	156·5	15	39·0	54·0
142·6	154·2	30	40·4	52·8
138·7	153·3	45	40·7	57·4
143·7	144·5	60	42·7	58·0
138·9	145·4	75	41·2	58·2
133·2	145·3	90	40·8	60·6
137·7	135·5	105	39·1	53·7
127·5	131·9	120	38·5	53·6
123·2	121·5	135	37·5	53·7
116·1	117·9	150	36·2	51·2
Averages for first hour (15′–30′–45′) and last hour (120′–135′–150′) of incubations:				
141·6 ± 7·8	154·7 ± 6·3 "first"		40·0 ± 7·3	54·7 ± 8·2
122·3 ± 3·3	123·7 ± 4·1 "last"		37·4 ± 4·9	52·8 ± 3·8
Tissue wet wt.: untreated: 102·1 mg ± 13·0				
treated: 102·1 mg ± 12·3				

* 70 μg of angiotensin added to the solution (7 ml) on the serosal side of one bladder half of each pair, every 15 min, throughout the incubation period.

II had on the average decreased, relative to the control membranes, by 11·7 μA; the difference however was not stastically significant (S.E. mean difference: 12·5; $p > 0{\cdot}3$). No definite influence on the transmembrane potential resulted from the treatment either. With this experimental setting the concentration of undegraded angiotensin II in the incubation fluid was never inferior to 5×10^{-5} M, as appears from assays with the rat preparation.

In 6 cases, both bladder halves of each pair were treated with aldosterone: after three hours of incubation, which allows for the steroid hormone to develop its full effect in the experimental conditions chosen,[20] 70 μg of angiotensin II were added every 15 min during one hour to the solution (7 ml) on the serosal side of one of the paired membranes. Again, there was a slight, albeit statistically not significant, decrease in sodium transport activity as expressed by short-circuit current, when the membranes had been exposed to angiotensin II (mean decrease 5·2 μA; S.E. mean difference: 5·0; $p > 0{\cdot}3$).

In 12 experiments the bladders treated with angiotensin were exposed to Pitressin together with the untreated matched membranes. The short-circuit current, thereafter read every 5 min for half an hour, increased on the average twice as much (Δ : 94 μA) for the bladder halves treated with angiotensin as did their paired control halves (Δ : 55 μA). The mean difference in response to Pitressin was 39 μA $\pm$ 13·8 ($p < 0{\cdot}02$).

DISCUSSION

The toad bladder and skin can be considered to a certain extent as models for study of the sodium transport activity of parts at least of the mammalian nephron. These biological membranes have already been shown to respond to hormones such as aldosterone and antidiuretic hormone. Yet neither the bladder nor the skin reacted to angiotensin II in a manner likely to throw light on the effects reported for the mammalian kidney, since there occurred with these preparations at most a slight decrease in sodium transport activity as a delayed response to high doses of angiotensin.

There was extensive inactivation of the material whenever it was assayed for pressor activity, a good index of the preservation of the structure of this molecule.[21] The present results, being at variance with those of Barbour *et al.*[22] are not surprising since several widespread enzyme systems are capable of breaking down this octapeptide,[23] and since angiotensinase activity has been found in toad plasma[24]—a fact confirmed in this laboratory. Care was thus taken to add angiotensin II at sufficiently frequent intervals for adequate appraisal of its effect on sodium transport which implied continuous presence of unaltered angiotensin. As said, no convincing effect was observed either on short-circuit current or on potential difference. The preparation was not made more responsive to angiotensin by exposure to aldosterone as could have been thought of on the basis of results of Laragh *et al.* in cirrhotics[7] and Croxatto *et al.*[3] in rats. In normal man however the effect of angiotensin II on sodium excretion is not modified by aldosterone.[25, 26]

It would be presumptuous to draw from these negative observations the conclusion that renal tubular sodium transport in mammals is not influenced by angiotensin II. Langford[27], perfusing chicken kidneys through the renal portal vein, concluded that there was an inhibitory effect exerted by angiotensin II on renal tubular transport of sodium. These results have been recently confirmed.[28] Evidence has also been

obtained of an inhibitory effect on proximal tubular sodium transport by means of studies on renal cortex slices[29] and on the intact rat,[30] while Vander[31] using the stop-flow method had come to the conclusion that in the dog there was inhibition of distal tubular sodium reabsorption instead. Such findings point at some direct interference on the part of angiotensin II with tubular sodium transport. Yet, a kidney Na–K sensitive ATPase, presumably involved in tubular sodium transport, is not influenced by angiotensin.[32]

One could surmise that angiotensin II exerts an effect solely through an increase in the passive transfer of sodium from peritubular fluid to lumen. Such an influence would result in an increased excretion of sodium without modification in the activity of the sodium "pump". But, everything being equal otherwise, such a "shunting" effect would result in a decreased transmembrane potential, and this was not found when angiotensin was tested on the toad skin and bladder.

The response of the short-circuit current to vasopressin noted when the toad bladder was exposed to angiotensin II is probably not due to a slower degradation of vasopressin in the presence of a large excess of the other octapeptide because the vasopressin effect was as short-lived in the treated and untreated membranes; furthermore, in human blood the degradation of both hormones proceeds quite independently.[33]

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