Biochemical Pharmacology, Vol. 25, pp. 106–107. Pergamon Press, 1976. Printed in Great Britain.

# Effect of angiotensin II on short-circuit current in amphibian membranes

(*Received* 10 *April* 1974; *accepted* 14 *March* 1975)

Evidence of an effect of angiotensin II on active transport of sodium in amphibian tissues has accumulated in the last years despite preliminary negative reports [1, 2]. Although angiotensin II has been found to stimulate the short-circuit current in frog skin [3] and sodium reabsorption in isolated toad kidney [4] and to produce a natriferic effect in the whole toad *Bufo paracnemis* [5], the lack of an explanation of the failure in previous experiments and the negative results with other preparations, like isolated renal tubules [6, 7], give the reader of reviews on the subject the impression of an unsettled question [8, 9].

It seems at present clearly demonstrated that angiotensin II increases sodium transport in isolated frog skin [3] and toad kidney [4] as well as in mammalian tissues [10–12]. The effect of angiotensin II does not seem to be restricted to sodium transport, since evidence of a significant increase in water permeability in the whole toad [5] and in isolated toad bladder [13] and skin [14] was obtained. In this paper we present experiments performed on skin of different species of toads in which angiotensin II stimulates short-circuit current; further evidence is provided that previous negative results may be explained on the basis of the great variability in responses for each group of animals. Although the cause of this variability remains to be settled, some factors that may affect it have been studied.

In the present set of experiments, short-circuit current (SCC) was measured by the method of Ussing and Zerahn [15] in the abdominal skin and bladder of different species of toads (*Bufo arenarum*, *Bufo paracnemis* and *Ceratophrys ornata*) and in the South American frog *Leptodactylus chaquensis*, in several seasons of the year. In the frog *Leptodactylus chaquensis*, it is possible that active transport of chloride plays a role, but that would not be the case for *Bufo arenarum* [16, 17].

Table 1 shows the effect of a single dose of angiotensin II added to the internal side of the skin in doses of $10^{-5}$ to $10^{-6}$ M. The increase in SCC or transmembrane potential was maximal at 15 min as compared to the last of three stable readings (Fig. 1). Table 1 demonstrates that frogs and toads react to angiotensin II and that the variability of response is not season dependent, since both reactive and nonreactive *Bufo paracnemis* toads are observed in summer. It is known that frog skin responses to antidiuretic hormone exhibit seasonal changes [18], but neither these nor changes in skin resistance seemed to account for the response to angiotensin II; this latter parameter did not vary significantly as compared to nonresponsive *Bufo paracnemis* skins in the same season. Furthermore, Fig. 1 shows that an experimental factor was involved in the insensitivity to angiotensin II exhibited by toad skin, since in some experiments performed in paired halves of abdominal skin only one half was seen to react.

Pretreatment with distilled water or saline (0·6% NaCl) had an effect on the SCC response to angiotensin II which could be attributed to an increase or decrease of basal current levels. The response is more easily observed after saline pretreatment, as has been demonstrated by Crabbé [19] for aldosterone. High basal SCC values result in the disappearance of the response to angiotensin II. Amphibians used in the experiments shown in Table 1 received no pretreatment. In spite of varying SCC levels, no significant differences were found in the baseline levels of reactive as compared to nonreactive groups, nor was any significant correlation found between the level of SCC of each skin and the magnitude of its response to angiotensin II.

In the data presented in Table 1, care was taken not to use toads which were moulting, since previous experiments measuring fluid uptake in the whole animal [5] demonstrated that the response to angiotensin II disappeared in moulting animals. It is known from Nielsen's experiments [20] that aldosterone-induced moulting causes inhibition of the skin response to this hormone. This is attributed to a hypothetical inhibitory substance released during the moulting process. The situation is not the same for angiotensin II. In fact, it seems to be the opposite,

Table 1. Effect of a single dose of angiotensin II on short-circuit current in ventral skin of several species of amphibia in different seasons*

| Species | Season | Dose (M) | N | Per cent increase† | | | |
|---|---|---|---|---|---|---|---|
| | | | | Transmembrane potential | P | Short-circuit current | P |
| *Leptodactylus chaquensis* | Summer | $10^{-5}$ | 6 | 20·3 ± 10·4 | NS | 30·3 ± 6·4 | <0·01 |
| | Autumn | $10^{-5}$ | 14 | 4·7 ± 1·9 | <0·05 | 9·3 ± 3·6 | <0·02 |
| *Bufo arenarum* | Summer | $10^{-5}$ | 6 | 2·6 ± 2·8 | NS | 4·0 ± 4·8 | NS |
| | Autumn | $10^{-6}$ | 8 | 6·2 ± 1·4 | <0·01 | 13·8 ± 4·2 | <0·02 |
| | Spring | $10^{-6}$ | 7 | 6·1 ± 1·8 | <0·02 | 14·4 ± 3·1 | <0·01 |
| *Bufo paracnemis* | Summer | $10^{-5}$ | 7 | 6·0 ± 2·2 | <0·05 | 13·0 ± 3·5 | <0·01 |
| | Summer | $10^{-5}$ | 6 | 3·4 ± 3·8 | NS | 8·1 ± 8·1 | NS |
| | Autumn | $10^{-5}$ | 9 | 6·0 ± 3·3 | NS | 3·4 ± 7·2 | NS |
| *Ceratophrys ornata* | Summer | $10^{-5}$ | 6 | 4·4 ± 0·8 | <0·01 | 6·5 ± 0·8 | <0·001 |

* Experiments were performed at 20°–27°.

† Per cent increase and S.E. of the reading at 15 min after adding angiotensin II and the last of three stable readings during the previous period. NS = not significant.

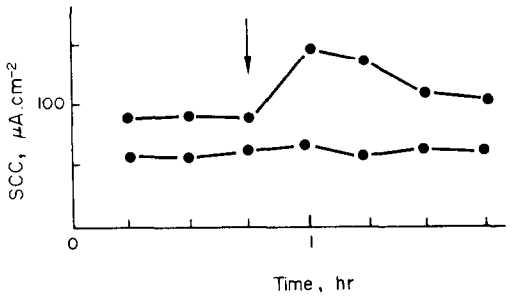


Fig. 1. Effect of angiotensin II ($10^{-5}$ M, arrow) on short-circuit current (SCC) in paired halves of an abdominal skin of *Ceratophrys ornata*.

since it has not been demonstrated that the octapeptide induces moulting, and skin reactivity actually increases when the *stratum corneum* is present. Supporting this conclusion, it can be mentioned that the reactivity to angiotensin II of toad skin is inhibited during the process of spontaneous moulting but is recovered the following day, when formation of a new *stratum corneum* has presumably begun. Since moulting is under endocrine control [21], experiments were performed in the bladder of toads whose skin had moulted. The results showed that reactivity of bladder epithelia to angiotensin II was not affected in this situation.

In previous experiments [2] failure of the skin to react to angiotensin II was attributed to inactivation of the peptide, and therefore doses were added every 15 min to maintain a high concentration without any stimulatory effect. In the present experiments angiotensin II-$^{125}$I was used to determine whether failure of some membranes to react was due to lack of binding to the receptor site of the membrane. It was demonstrated that angiotensin II had not been adsorbed in the reservoirs and had reached the membrane, which in turn had failed to react. Membranes pretreated with angiotensin II showed a greater reactivity to vasopressin in previous experiments; this fact at present is tentatively explained on the basis that the adenylate cyclase–cyclic AMP system could be involved in the response to angiotensin II [22].

Failure of some groups of toads may also reflect problems derived from amphibian experimentation, as Herrera [23] pointed out in referring to the discrepancy in results obtained from toads of Colombian and Dominican origin, whereas Culley [24] and Gibbs *et al.* [25] claimed to develop definite strains of amphibians to avoid inconsistent results from experimentation. Inconsistency in the reactivity of amphibian membranes to angiotensin II remains to be fully explained, although it should be pointed out that these tissues react to a very small extent (up to about 15 per cent of the maximal response) to neurohypophyseal peptides and effective doses ($10^{-6}$ M) are higher than those used in mammalian membranes ($5{\cdot}10^{-10}$ M) [12].

If angiotensin II is able to stimulate active sodium transport either by increasing sodium permeability or by stimulating the sodium pump, its action not only in the renal tubule but also in other systems (the arterial wall [26], brain tissue [27], norepinephrine uptake in the sympathetic nerve endings and aldosterone secretion by the adrenal cortex [28]) could be explained on the basis of changes in membrane permeability or sodium pump activity, as suggested by other authors.

*Acknowledgements*—The authors are indebted to Doctor Jean Crabbé (University of Louvain, Belgium) for his comments and to Mr. E. Rothe for correction of the English manuscript. These investigations were supported by grants of the Consejo Nacional de Investigaciones Científicas (5588/72) and the Subsecretaría de Ciencia y Técnica (051-L 44) de la República Argentina. Doctors P. Desaulles and K. Scheibli (CIBA-GEIGY, Basel, Switzerland) generously provided angiotensin II.

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