## SHORT COMMUNICATIONS

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### The effect of angiotensin on renal carbonic anhydrase

There has been considerable interest in recent years in the possibility that angiotensin may inhibit sodium transport by renal tubules. It is known that in whole animal experiments, in a variety of species, including the rabbit, angiotensin can cause natriuresis[1-5]. Most authors have speculated that this increased sodium excretion results from decreased sodium reabsorption by the tubules, and stopped-flow studies in the dog have supported the concept that angiotensin has a direct effect on tubular transport of sodium[6]. However, whole animal experiments do not exclude the alternative explanation that the observed natriuresis is the result of altered intra-renal haemodynamics, and not the result of a direct tubular action. In fact, direct evidence for an effect of angiotensin on sodium transport mechanisms is lacking. Angiotensin has been shown to have no effect on ($Na^+$-$K^+$)-activated ATPase from rabbit kidney[7], and it had no effect on sodium transport by the toad skin[8]. An early report that angiotensin inhibited sodium transport in kidney tissue slices[9] has also been refuted[10]. An answer should be obtained to this question, since an effect of angiotensin on sodium transport by renal tubules is an important part of current hypotheses regarding the nature of glomerular filtration[11] and renal auto-regulation[12].

Another possible way in which angiotensin might influence sodium reabsorption by the kidney is by inhibition of renal tubule carbonic anhydrase (carbonate hydrolyase, EC 4.2.1.1). This enzyme promotes sodium reabsorption by providing $H^+$ for exchange with $Na^+$. It was therefore decided to prepare carbonic anhydrase from rabbit kidney cortex and medulla, and to assess whether angiotensin has any inhibitory effect on this enzyme.

To prepare carbonic anhydrase, rabbits were killed by carotid exsanguination, and the kidneys promptly removed and flushed with 50 ml of 1 M NaCl *via* the renal artery, to remove red cells. The kidneys were then cut in sections, and the cortex dissected from the medulla with scissors. Cortex and medulla were then weighed, and ground separately with a mortar and pestle, during which 3 ml of glass distilled water per g of kidney tissue were added. After filtering through gauze, the extract was stored frozen, or dialysed for 24 h at 4° against glass distilled water, and then stored frozen.

Carbonic anhydrase activity of the extracts was assayed by the indicator technique of ROUGHTON AND BOOTH[13]. In this technique, water saturated with $CO_2$ is added to the sample to be tested in the presence of Veronal buffer (pH 8.2) and bromothymol blue indicator. The time taken in seconds for a fall in pH to 6.3, as shown by a change in colour of the indicator, is used as a measure of carbonic anhydrase activity. All estimations are carried out at 0° by surrounding the samples with crushed ice. It was found that cortical extracts required 1:10 dilution with water, since carbonic anhydrase activity was very high, but medullary extracts were not diluted. The experiment was set up with water blanks, asparginyl valine-5 angiotensin (Hypertensin, Ciba) blanks, enzyme controls, and tubes containing enzyme and angiotensin, the latter in final concentrations of $8.6 \cdot 10^{-2}$ mM down to $8.6 \cdot 10^{-6}$ mM angiotensin. As a

check on the validity of the preparation, acetazoleamide was added to some enzyme-containing tubes; this produced 89% inhibition of carbonic anhydrase activity in a concentration of 0.01 mM.

Because kidney extracts prepared in this way may contain angiotensinases, which might destroy added angiotensin, some measure of the persistence of angiotensin activity at the end of the carbonic anhydrase assay was necessary. Therefore samples from the carbonic anhydrase assay at each angiotensin concentration used were added to a rabbit jejunum preparation for the bio-assay of angiotensin. This preparation is in standard use in our laboratory. Angiotensin causes contraction of the intestine, which is magnified by a lever; the response to angiotensin standards between 30 and 300 ng/100 ml is linear, and responses are measurable down to 15 ng/100 ml angiotensin. Angiotensin bio-assays were done in the case of the blank and enzyme control tubes, as well as the angiotensin tubes.

It was found, in fact, that angiotensin activity was little affected under the assay conditions. At the end of the assay, the mean remaining angiotensin activity for all concentrations used was 96.3% of the amount initially added (range 83.4 to 104.8%). The blank caused a small contraction of the intestine, probably because of its low temperature, since warming removed this effect. Apart from this minor temperature effect, samples from the enzyme control tubes caused no effect on the bio-assay, so that it was assumed that negligible amounts of angiotensin were initially present in the extract.

Dialysis of the enzyme extract made no difference to the result of the carbonic anhydrase assay. Presumably removal of low molecular weight substances by dialysis did not alter carbonic anhydrase activity. It was also noted that enzyme concentration was linearly related to the reciprocal of the time in sec taken for the carbonic anhydrase assay, even when control times were as low as 4% of the blank times. Further, angiotensin added to the tubes in the absence of enzyme (angiotensin blanks) had no effect on the time of reaction (blank time); and angiotensin, in the concentrations used, added to the buffer did not alter its pH.

TABLE I

THE EFFECT OF ANGIOTENSIN ON RABBIT RENAL CARBONIC ANHYDRASE FROM CORTEX AND MEDULLA

Each result is the mean of 4–16 readings. Differences in times between control tubes containing carbonic anhydrase, and those tubes with carbonic anhydrase *plus* angiotensin, are not significant, but the differences between blanks and controls are all significant ($P < 0.001$). The numbers in parentheses refer to the numbers of tests run.

| *Concn. of added angiotensin (mM)* | *Cortex* | | | *Medulla* | | |
|---|---|---|---|---|---|---|
| | *Mean blank time (sec)* | *Mean control (carbonic anhydrase) time (sec)* | *Mean carbonic anhydrase + angiotensin (sec)* | *Mean blank time (sec)* | *Mean control (carbonic anhydrase time (sec)* | *Mean carbonic anhydrase + angiotensin (sec)* |
| $8.6 \cdot 10^{-2}$ | 174.9 | 21.3 | 21.1 (13) | 159.4 | 16.2 | 14.7 (4) |
| $8.6 \cdot 10^{-3}$ | 186.6 | 23.3 | 23.8 (16) | 159.4 | 16.2 | 14.9 (8) |
| $8.6 \cdot 10^{-4}$ | 186.6 | 23.3 | 23.5 (16) | 159.4 | 16.2 | 15.3 (8) |
| $8.6 \cdot 10^{-5}$ | 186.6 | 23.3 | 23.5 (16) | 159.4 | 16.2 | 16.3 (8) |
| $8.6 \cdot 10^{-6}$ | 167.0 | 22.3 | 22.0 (12) | 159.4 | 16.2 | 15.6 (8) |

The results are summarised in Table I. Both dialysed and undialysed enzyme results (done in equal numbers of experiments) are combined in Table I, since dialysis made no difference. It was found that angiotensin, in the amounts added, had no effect on the activity of renal carbonic anhydrase from either cortex or medulla. The greatest difference in time between any enzyme control value and any of the 109 tubes containing angiotensin at all concentrations studied was 7.3 sec, compared with an average difference of 153 sec between enzyme controls and blanks.

Because it was still possible that angiotensin may inhibit carbonic anhydrase at body temperature, further assays were done after pre-incubation of angiotensin with the kidney extract at 37° for 45 min. Angiotensin was added in four concentrations, ranging from $8.6 \cdot 10^{-4}$ to $8.6 \cdot 10^{-2}$ mM. However, no inhibition was seen (mean control time 32.1 $\pm$3.5 sec, mean time of angiotensin-treated samples 29.3 $\pm$1.8 sec, mean blank time 231.7 $\pm$10.5 sec). Incubation at 37° for 45 min did not influence the results of control assays. Under these circumstances, some loss of angiotensin activity was found; average residual activity, assessed from the intestine bio-assay, was 80.5% of the amount of angiotensin initially added for all concentrations used (range 69.1 to 91.8%).

It was concluded that angiotensin does not inhibit carbonic anhydrase in the rabbit kidney cortex or medulla, in the range of the concentrations used. Taken in conjunction with previous studies of BONTING, CANADY AND HAWKINS[7], of the effect of angiotensin on rabbit kidney ($Na^+$-$K^+$)-activated ATPase, the data suggest that the natriuresis induced by angiotensin does not appear to be due to inhibition of either of these enzyme systems.

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*Lions Renal Research Laboratory,*
*Princess Alexandra Hospital,*
*South Brisbane, Queensland*
*(Australia)*

JOHN K. HEALY
JON B. DOUGLAS

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