*J. Pharm. Pharmac.*, 1975, 27, 193–196 *Received August* 16, 1974

# How does angiotensin II increase cardiac dopamine-β-hydroxylation?

CLAUDE CHEVILLARD, NICOLE DUCHENE AND JEAN MICHEL ALEXANDRE
*Centre de Recherche sur l'Hypertension Artérielle*
AND
*Département de Pharmacologie de la Faculté de Médecine Broussais-Hôtel-Dieu, Hôpital Broussais, 96 rue Didot, Paris, France*

The potent accelerating effect of angiotensin II (Ang II) on cardiac dopamine β-hydroxylation was studied on slices of rat heart. Ang II did not affect the kinetics of β-hydroxylation but it increased the axonal uptake of dopamine, and, concomitant with the acceleration of biosynthesis, it enhanced the accumulation of dopamine into tissue. Puromycin, in contrast to actinomycin D, antagonized the stimulation of dopamine β-hydroxylation by Ang II, but did not suppress the rise in cardiac dopamine. Therefore, to promote the acceleration of dopamine β-hydroxylation, (i) the rise in tissue dopamine available for conversion appeared to be insufficient, (ii) the formation of new proteins by activation of traduction seemed to constitute the basic mechanism of Ang II action.

Angiotensin II (Ang II) has been reported to alter the functioning of postganglionic sympathetic fibres. The drug did not change the axonal uptake of noradrenaline (Chevillard & Alexandre, 1970; Hughes & Roth, 1971; Trager, Kreye & Gross, 1972), but was shown to release newly synthesized noradrenaline and to accelerate the transformation of dopamine into noradrenaline (Chevillard, Duchene & Alexandre, 1971). However, the mechanism of these changes is as yet unknown. We have sought the mechanism by which Ang II accelerates dopamine β-hydroxylation. Three mechanisms might be involved: (i) an increase of the enzyme reaction rate; (ii) a greater amount of dopamine available for β-hydroxylation, and, (iii) an enhancement in the synthesis of dopamine β-hydroxylase (DBH) or other proteins required for β-hydroxylation.

## MATERIALS AND METHODS

The experiments were on slices of rat heart. Ang II (Angiotensin II amide; Ciba Corporation) was used at a concentration ($5 \cdot 10^{-6}$M) which has been previously reported to increase dopamine β-hydroxylation (Chevillard, Duchene & Alexandre, 1971).

### *Influence of Ang II on kinetics of β-hydroxylation*

The effect of Ang II on the rate of conversion of several concentrations of tyramine into octopamine, by cardiac DBH was examined. Octopamine was estimated by the method of Goldstein, Freedman & Bonnay (1971) after transformation into its *N*-methylated derivative, [$^{14}$C]synephrine.

Ventricles of rats were homogenized in 0·05M tris buffer, pH 6·8, containing 0·1% Triton X 100. After centrifugation (50 000 g, 20 min, 0°), aliquots (0·1 ml) of the supernatant were incubated (20 min, 37°, pH 6) with tyramine (5 to 50 nM).

A second incubation (pH 8·6, 37°, 30 min) was made in presence of *S*-adenosyl-[$^{14}$C]methionine (0·05 $\mu$Ci, 20 mCi m mol$^{-1}$, C.E.A., France) and of partially purified P.N.M.T. (Axelrod, 1962), both in excess, in order to transform octopamine into [$^{14}$C]synephrine. After adjusting the pH to 11, [$^{14}$C]synephrine was extracted with a mixture of toluene and isoamyl alcohol (3:2 v/v) and estimated by liquid scintillation counting.

Results were expressed in n mol of octopamine formed per g of wet tissue, per hour. A double reciprocal plot (Lineweaver & Burke) was used to analyse the influence of Ang II on the kinetics of $\beta$-hydroxylation.

*Influence of the synthesis of proteins on the increase in dopamine $\beta$-hydroxylation by Ang II*

Effects of actinomycin D and puromycin on the activation by Ang II of dopamine $\beta$-hydroxylation were sought.

Ring tritiated dopamine (200 mCi mmol$^{-1}$) (Radiochemical Centre, Amersham, England) was used to obtain newly synthesized noradrenaline in slices of rat ventricle. Slices (2500 mg, wet weight) were incubated for 90 min at 37° in oxygenated Krebs solution with [$^{3}$H]dopamine ($^{3}$H-DA) (25 $\mu$Ci) and pargyline (1·25 $10^{-4}$M). Slices were then washed and reincubated for 90 min with Ang II, and/or with actinomycin D or puromycin (1·$10^{-4}$M).

Newly synthesized [$^{3}$H]noradrenaline ($^{3}$H-NA), and $^{3}$H-DA were determined in the tissue and in the incubation medium. Separation of both catecholamines was by alumina adsorption and ion-exchange chromatography (Dowex AG.50.$WX_4$.$Na^+$) (Glowinski, Iversen & Axelrod, 1966). Average recoveries for $^{3}$H-NA and $^{3}$H-DA were 75 and 65%, respectively. Contamination of $^{3}$H-NA by $^{3}$H-DA never exceeded 5%. All data were corrected for recovery and for a contamination of 5%.

*Action of Ang II on the uptake of $^{3}$H-DA by the heart*

Slices (200 mg, wet weight) of ventricle of rat were incubated for 5 min, in 10 ml of oxygenated Krebs solution containing 25 nCi of $^{3}$H-DA and Ang II. Tissue $^{3}$H-DA, was isolated and estimated, as above.

Student's *t*-test was used to ascertain the results values $\pm$ s.e.m.

## RESULTS

*Influence of Ang II on kinetics of $\beta$-hydroxylation*

The Lineweaver-Burke plot revealed that Ang II did not alter either the affinity of cardiac $\beta$-hydroxylase for tyramine or the maximal velocity of the reaction.

*Influence of the protein synthesis on the activation by Ang II of dopamine $\beta$-hydroxylation*

Table 1 shows that Ang II markedly increased, on one hand, the amount of newly synthesized noradrenaline recovered in the incubation medium, and, on the other hand, cardiac $^{3}$H-DA. The sum of $^{3}$H-NA found in the tissue and in the bath was greater in the presence of Ang II than in the control experiments. Therefore, Ang II accelerated the synthesis of $^{3}$H-NA from cardiac $^{3}$H-DA.

Actinomycin D and puromycin did not affect the basal rates of noradrenaline release and synthesis or $^{3}$H-DA distribution. Actinomycin D did not affect the

Table 1. *The influence of angiotensin II, puromycin and actinomycin D on the efflux of newly synthesized noradrenaline, on its synthesis, and the tissue accumulation of dopamine in cardiac slices.*

| | | $^3$H- NA newly synthesized nCi g$^{-1}$ | | | $^3$H-DA nCi g$^{-1}$ | |
|---|---|---|---|---|---|---|
| | | T | M | T + M | T | M |
| Controls | (6) | 195 ± 11 | 94 ± 9 | 289 ± 19 | 353 ± 11 | 389 ± 23 |
| Ang II 5·10$^{-6}$M | (7) | 218 ± 15 | 174 ± 11*** | 392 ± 20** | 508 ± 23*** | 283 ± 17*** |
| Actinomycin D 1·10$^{-4}$M | (6) | 188 ± 10 | 92 ± 8 | 280 ± 12 | 370 ± 9 | 392 ± 16 |
| Puromycin 1·10$^{-4}$M | (7) | 207 ± 11 | 86 ± 8 | 293 ± 17 | 342 ± 12 | 371 ± 14 |
| Ang II + actinomycin D | (7) | 197 ± 15 | 193 ± 10*** | 390 ± 19** | 549 ± 16 | 398 ± 18 |
| Ang II + puromycin | (7) | 110 ± 14† | 159 ± 7*** | 269 ± 22† | 558 ± 21 | 375 ± 21 |

Values are the mean and s.e.m.—(N) No of experiments.
† $P < 0{\cdot}01$ compared to the Ang II value.
** $P < 0{\cdot}01$; *** $P < 0{\cdot}001$ compared to the control value.
T: Amount of $^3$H amines in cardiac slices.
M: Amount of $^3$H amines in the incubation medium.

stimulatory actions of Ang II. However, puromycin abolished the increase in noradrenaline biosynthesis but failed to abolish the rise in tissue $^3$H-DA and in $^3$H-NA release which is normally induced by Ang II.

*Action of Ang II on uptake of $^3$H-DA by the heart*

Ang II increased the incorporation of $^3$H-DA into cardiac slices during a 5 min incubation period (control mean ± s.e.m. 3·25 ± 0·37 nCi g$^{-1}$ $^3$H-DA n = 10; Ang II 5 × 10$^{-6}$M 5·86 ± 0·63, n = 10 $P < 0{\cdot}01$).

## DISCUSSION

Ang II was able to enhance the biosynthesis of noradrenaline from dopamine. Such an action was not the result of change in the kinetics of the reaction: neither the affinity of DBH for its substrate, nor the maximal velocity of β-hydroxylation were increased by Ang II. Since actinomycin D did not change the acceleration of dopamine β-hydroxylation induced by Ang II, whereas puromycin inhibited this effect, it appears that Ang II acted upon protein synthesis by activating messenger-RNA traduction. Whether Ang II increased DBH synthesis or induced the formation of other proteins, such as carrier proteins necessary for vesicular incorporation of dopamine, could not be ascertained from these experiments.

Supportive documentation for the present analysis first comes from observations of Boadle-Biber, Hughes & Roth (1972) who reported that Ang II enhanced another limiting step of noradrenaline synthesis: tyrosine hydroxylation. They also showed that cycloheximide and puromycin inhibited Ang II action. On the other hand, it is well known that some effects of Ang II, such as aldosterone production (Cosby, Roth & Sartorelli, 1971) and increase in transfer of sodium and water across the mucous membrane of colon (Davies, Munday & Parsons, 1969), required the formation of new proteins. As in our experiments, actinomycin D did not change these actions of Ang II, but puromycin abolished them.

Concomitant with the acceleration of dopamine β-hydroxylation, Ang II caused the release of newly synthesized noradrenaline. Is the increase in β-hydroxylation

the result of that release? In fact, previously reported data concerning the action of both tyramine and amphetamine (Chevillard & Alexandre, 1972) were inconsistent with such a relation, since both amines, in spite of their releasing effect on noradrenaline stores, did not increase, but inhibited dopamine $\beta$-hydroxylation.

Nevertheless, there is an important difference between the effects of amphetamine and tyramine on one hand and those of Ang II on the other; the indirectly acting sympathomimetic amines reduced the content of dopamine in cardiac slices, but that content was increased by Ang II. These data fully suggests that tissue amounts of dopamine available for $\beta$-hydroxylation may control the reaction rate.

Such an increase in tissue dopamine concentration appears, however, to be insufficient to promote an acceleration of dopamine $\beta$-hydroxylation, since puromycin did not prevent the rise in tissue dopamine induced by the peptide, but was able to antagonize the stimulatory action of Ang II on noradrenaline biosynthesis.

Therefore, new proteins have to be synthesized for acceleration of dopamine $\beta$-hydroxylation by Ang II. Such a new protein might be DBH itself, directly induced by Ang II, or, indirectly by the rise in dopamine near the $\beta$-hydroxylation sites.

*Acknowledgements*

The authors gratefully acknowledge the assistance of a grant from the Institut National de la Santé et de la Recherche Médicale.

## REFERENCES

AXELROD, J. (1962). *J. biol. Chem.*, **237**, 1657–1660.
BOADLE-BIBER, M. C., HUGHES, J. & ROTH, R. G. (1972). *Br. J. Pharmac.*, **46**, 289–299.
CHEVILLARD, C. & ALEXANDRE, J. M. (1970). *Experientia*, **26**, 1334–1336.
CHEVILLARD, C., DUCHENE, N. & ALEXANDRE, J. M. (1971). *Eur. J. Pharmac.*, **15**, 8–14.
CHEVILLARD, C. & ALEXANDRE, J. M. (1972). *Ibid.*, **19**, 223–230.
COSBY, L. A., ROTH, R. H. & SARTORELLI, A. E. (1971). *Pharmacologist*, **13**, 126.
DAVIES, N. T., MUNDAY, K. A. & PARSONS, B. J. (1969). *J. Physiol. (London)*, **205**, 17P–19P.
GLOWINSKI, J., IVERSEN, L. L. & AXELROD, J. (1966). *J. Pharmac. exp. Ther.*, **151**, 385–399.
GOLDSTEIN, M., FREEDMAN, S., & BONNAY, M. (1971). *Experientia*, **27**, 632–633.
HUGHES, J. & ROTH, R. H. (1971). *Br. J. Pharmac.*, **41**, 239–255.
TRAGER, E., KREYE, V. A. W. & GROSS, F. (1972). *Arch. Pharmac.*, **274**, 203–207.