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Brain tyrosine hydroxylase activity and systolic blood pressure in rats treated with either deoxycorticosterone and salt or angiotensin

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The hypertension induced in adult male rats by doca/salt was found to be accompanied by a significant rise in whole brain tyrosine hydroxylase (TH) activity. A smaller hypertensive effect, produced by angiotensin (750 ng kg^{-1} daily) was also accompanied by a proportional rise in whole brain TH activity. The specific antagonists spironolactone and saralasin completely blocked both responses in the doca/salt- and angiotensin-treated animals respectively and spironolactone showed a partial inhibition of the effects of angiotensin. In all the animals treated there was a clear correlation between systolic blood pressure and whole brain TH activity. The significance of these changes is discussed in the light of the central mechanism of hypertension.

Increasing attention is now being directed to the role of brain catecholamines in the control of the sympathetic regulation of blood pressure (Chalmers, 1975). The rate-limiting enzyme in catecholamine biosynthesis, tyrosine hydroxylase (TH) (Levitt, Spector, & others, 1965) has been shown to increase activity in sympathetic ganglia, adrenal medulla and brain stem of rats after the administration of reserpine (Mueller, Thoenen & Axelrod, 1969a) and in the adrenal gland alone after 6-hydroxydopamine and phenoxybenzamine (Thoenen, Mueller & Axelrod, 1969). Mueller, Thoenen & Axelrod (1969b) have proposed that the increased activity of this enzyme under these conditions is a result of increased efferent nerve activity and, since the reserpine-induced increase in TH activity in the adrenal medulla and sympathetic ganglia of the rat can be prevented by the administration of cycloheximide or actinomycin D, that enzyme synthesis is regulated by release or turnover of catecholamines.

De Champlain, Krakoff & Axelrod (1969a) and De Champlain, Mueller & Axelrod (1969b) have shown increased noradrenaline turnover in sympathetically innervated tissues during deoxycorticosterone (doca)/salt-induced experimental hypertension and Lee (1969), Dickinson & Ferrario (1974) and McCubbin (1974) have postulated a relation between the sympathetic nervous system and renal pressor mechanisms. Angiotensin appears to modulate noradrenaline release (Peach, 1974) and to have specific effects on the area postrema of the hind brain (McCubbin, 1974) in addition to its normal pressor action. Furthermore Scroop, Katic & others (1975) have suggested that angiotensin has a significant central role in renal hypertension in the dog.

Preliminary studies (Rylett, Dean & Lee, 1975) have shown that the administration of doca/salt or angiotensin causes increased TH activity in rat brain. We now report that these increases and the associated rise in systolic blood pressure can be blocked by specific antagonists and there is a clear correlation between systolic blood pressure and whole brain TH activity.

MATERIALS AND METHODS

Male Wistar rats, 200–250 g were kept in groups of six. Food and drink were freely available to all the animals; for animals that were receiving doca, water was replaced by 1% NaCl.

Val_5 angiotensin II amide (Ciba Batch 121710) 0·75 $\mu g\ kg^{-1}$ as 1·5 $\mu g\ ml^{-1}$ solution in normal saline was administered subcutaneously daily for 14 days; control groups received saline alone. Saralasin (1-Sar-8-Ala-angiotensin II) acetate (gift from Norwich Pharmacal Co) was prepared as a 30 $\mu g\ ml^{-1}$ solution in normal saline and administered once daily at 15 $\mu g\ kg^{-1}$, subcutaneously, simultaneously with angiotensin. All solutions of peptides were prepared in polyethylene or polystyrene containers and stored at −20°. Doca (Organon) 5 mg ml^{-1} in oil was administered at 12·5 mg kg^{-1} (s.c.) once every three days for fifteen days. Control animals received arachis oil. Spironolactone (Batch 124 gift, from G. D. Searle & Co Ltd)

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was prepared as a 500 mg ml^{-1} suspension in 5% acacia mucilage and administered at 250 mg kg^{-1} by stomach tube at the same time as the doca. Systolic blood pressure was measured regularly throughout the experimental period using the tail cuff method.

One day after the last treatment with the drug, or as required for zero controls, the rats were killed by stunning and cervical dislocation. The brains were immediately removed, chilled to 0° and stored at −20°. Brains were homogenized in 5 volumes of glass distilled water using a Tri-R Teflon glass homogenizer. 3 and 2 × 100 μl samples of this homogenate were used in protein and tyrosine hydroxylase activity determinations respectively.

TH activity was measured by the coupled decarboxylation of 3,4-dihydroxyphenylalanine (dopa) formed from tyrosine [1-^{14}C] (NEN GmbH, Germany) by the method of Waymire, Bjur & Weiner (1971). The coupling enzyme, dopa decarboxylase, was prepared from pig renal cortex and checked regularly for activity with DL-dopa [1-^{14}C] (Radiochemical Centre, Amersham, U.K.). The trapped $^{14}CO_2$ was counted in 10 ml of 0·6% 2-[4′-t-butylphenyl-5-(4″-biphenyl)1,3,4-oxadiazole] (butyl-PBD) (Hopkins & Williams Ltd) in redistilled toluene using a Packard 3320 counter with external standard channels ratio quench correction (efficiency 78–82%, 10 000 counts recorded).

Protein concentration was measured in triplicate according to Lowry, Rosebrough & others, (1951) and tyrosine hydroxylase activity expressed as μmol $^{14}CO_2$ mg^{-1} protein min^{-1}.

Tests for significance of TH activity or blood pressure changes during treatment were made by comparing test with control using Student's *t*-test.

RESULTS

The effect of spironolactone on the blood pressure rise caused by doca/salt is shown in Fig. 1. The hypertensive effect of doca/salt is evident 7 days after the start of treatment and the 31% rise is highly significant ($P > 0{\cdot}001$) on day 15. Administration of 250 mg kg^{-1} of spironolactone with doca/salt prevents the blood pressure rise. Table 1 summarises these results at the end of the treatment and shows that the rise in blood pressure following doca/salt is accompanied by a significant ($P < 0{\cdot}01$) rise in whole brain TH activity. The remaining treatments, including spironolactone alone, caused no significant rise in blood pressure or TH activity.

When animals were treated with angiotensin daily there was again a clear rise in systolic blood pressure from the 7th day of treatment leading to a significant

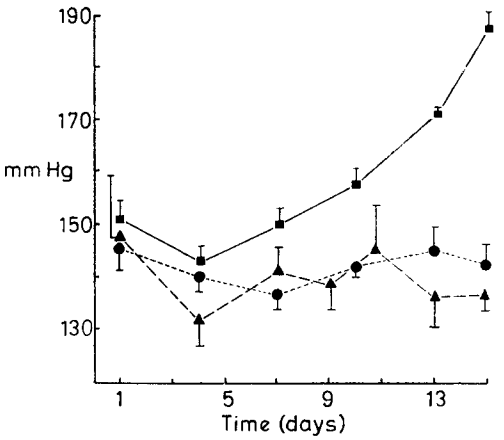

FIG. 1. Change in the systolic blood pressure of rats (mm Hg) (mean ± s.e. n = 6) during the administration of arachis oil (2·5 mg kg^{-1}, s.c. once every 3 days) ●- - - -●; Doca (12·5 mg kg^{-1}, 5 mg ml^{-1} in oil s.c. once every 3 days) and salt (1% NaCl as drinking fluid) ■——■; doca and salt (as above) together with spironolactone (250 mg kg^{-1}, 500 mg ml^{-1} in 5% acacia mucilage orally once every 3 days) ▲— —▲.

difference on day 14 ($P < 0{\cdot}01$) (Fig. 2). Simultaneous administration of saralasin with the angiotensin did not prevent this increase when the doses were equal and only reduced the rise when there was a 10 mol excess of the antagonist. A 20 fold excess of antagonist completely blocked this hypertensive effect of angiotensin. The whole brain TH activities corresponding to these treatments are shown in Table 2, the activity for angiotensin-treated animals being the only one significantly different from control, at the same time showing a valid difference from angiotensin + 20 × saralasin and 20 × saralasin alone (all $P < 0{\cdot}001$).

Spironolactone showed a partial inhibition of both the blood pressure and TH activity increase caused

Table 1. *Whole brain tyrosine hydroxylase activity and systolic blood pressure of rats after 15 days treatment with doca/salt and spironolactone.*

Treatment	Tyrosine hydroxylase activity mean±s.e. × 10^{-6} n = 6	% increase over control	*P*	Systolic blood pressure mean ±s.e. n = 6	% increase over control	*P*
I	1·24±0·01	—	—	144±3	—	—
II	1·80±0·07	45	<0·01	188±3	31	<0·001
III	1·22±0·09	—	N.S.	138±3	—	N.S.
IV	1·20±0·06	—	N.S.	139±6	—	N.S.

I. Arachis oil 2·5 mg kg^{-1} (s.c.) once every 3 days. II. Doca 12·5 mg kg^{-1}, 5 mgml^{-1} in oil (s.c.) once every 3 days and salt 1% NaCl to drink. III. Doca 12·5 mg kg^{-1}, 5 mg ml^{-1} in oil (s.c.) once every 3 days and salt 1% NaCl to drink. Spironolactone−250 mg kg^{-1}, 500 mg ml^{-1} in 5% acacia mucilage orally once every 3 days. IV. Spironolactone−250 mg kg^{-1}, 500 mg ml^{-1} in 5% acacia mucilage orally once every 3 days and salt 1% NaCl to drink.

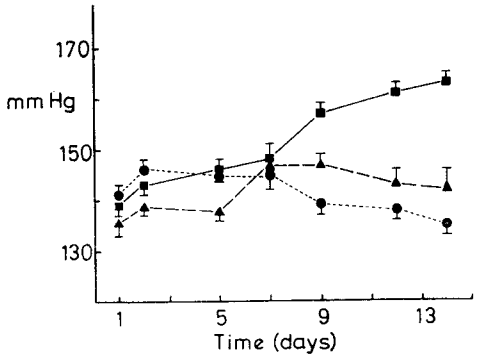

FIG. 2. Change in the systolic blood pressure of rats (mm Hg) (mean ± s.e. n = 6) during the administration of saline (0·5 mg kg^{-1}, s.c. daily) ●----●; angiotensin (0·75 μg kg^{-1}; 1·5 mg ml^{-1} in normal saline s.c. daily) ■——■; angiotensin (as above) and saralasin (7·5 μg kg^{-1}, 15 mg ml^{-1} in normal saline s.c. daily) ▲— — ▲.

by angiotensin (Table 3). The response to angiotensin was significantly higher ($P < 0·01$) than that to angiotensin plus spironolactone but spironolactone did not completely prevent either the rise of blood pressure or the change in TH activity.

Fig. 3 shows a plot of whole brain TH activity against systolic blood pressure for all groups of rats, control, agonist treated, agonist + antagonist and antagonist alone. This indicates a clear correlation between whole brain TH activity and systolic blood pressure (r = 0·874 $P < 0·001$ for 11d.f.).

DISCUSSION

The results reported confirm that in rats the hypertension which follows treatment with doca and salt (Tobian 1960) is associated with an increase in whole brain TH activity (Rylett, Dean & Lee, 1975). Kagawa, Sturtevant & van Arman (1959) have

Table 2. *Whole brain tyrosine hydroxylase activity and systolic blood pressure of rats after 15 days treatment with angiotensin and saralasin.*

	Tyrosine hydroxylase activity			Systolic blood pressure		
Treatment	mean±s.e. ×10^{-6} n = 6	% increase over control	P	mean ±s.e. n = 6	% increase over control	P
V	1·26±0·03	—	N.S.	142±2	—	N.S.
VI	1·29±0·04	—	—	135±2	—	—
VII	1·51±0·02*†	17·1	<0·001	163±2	21	<0·01
VIII	1·34±0·02*	4·4	N.S.	142±4	—	N.S.
IX	1·36±0·02†	5·4	N.S.	142±2	—	N.S.

* $P<0·001$ † $P<0·001$

V. None. VI. Saline—0·5 mg kg^{-1} (s.c.) daily. VII. Angiotensin—0·75 μg kg^{-1}, 1·5 μg ml^{-1} in normal saline (s.c.) daily. VIII. Angiotensin—0·75 μg kg^{-1}, 1·5 μg ml^{-1} in normal saline (s.c.) daily and saralasin—15 μg kg^{-1}, 30 μg ml^{-1} in normal saline (s.c.) daily. IX. Saralasin—15 μg kg^{-1}, 30 μg ml^{-1} in normal saline (s.c.) daily.

Table 3. *Whole brain tyrosine hydroxylase activity and systolic blood pressure of rats after 15 days treatment with angiotensin and spironolactone.*

	Tyrosine hydroxylase activity			Systolic blood pressure		
Treatment	mean±s.e. ×10^{-6} n = 6	% increase over control	P	mean ±s.e. n = 6	% increase over control	P
X	1·23±0·01	—	—	150±3	—	—
XI	1·46±0·02*	18·7	<0·001	167±4	11·3	<0·01
XII	1·37±0·02*	11·4	<0·001	163±4	8·7	<0·02
XIII	1·26±0·02	—	N.S.	155±3	—	N.S.

* These two results significantly different $P<0·01$.

X. Saline—0·5 ml kg^{-1} (s.c.) daily. XI. Angiotensin—0·75 μg kg^{-1}, 1·5 mg ml^{-1} in normal saline (s.c.) daily. XII. Angiotensin—0·75 μg kg^{-1}, 1·5 mg ml^{-1} in normal saline (s.c.) daily and spironolactone—250 mg kg^{-1} 500 mg ml^{-1} in 5% acacia mucilage orally once every 3 days. XIII. Spironolactone—250 mg kg^{-1}, 500 mg ml^{-1} in 5% acacia mucilage orally once every 3 days.

reported that spironolactone blocks the hypertensive effect of doca/salt and reduces similarly induced metacorticoid hypertension. In this work we have shown that spironolactone blocks both the hypertensive effect of doca/salt and the associated brain TH activity increase.

Muirhead, Leach & Armstrong (1973) have reported blood pressure increases following the daily administration of angiotensin, at 100μg kg^{-1} to unilaterally nephrectomized rats maintained on 1% NaCl. We did not anticipate a hypertensive response from the discontinuous administration of angiotensin alone to intact rats at a much lower dose. What is more interesting however is that we have also shown a corresponding rise in whole brain TH activity. Once this effect had been established the blocking effect of saralasin could be predicted but it

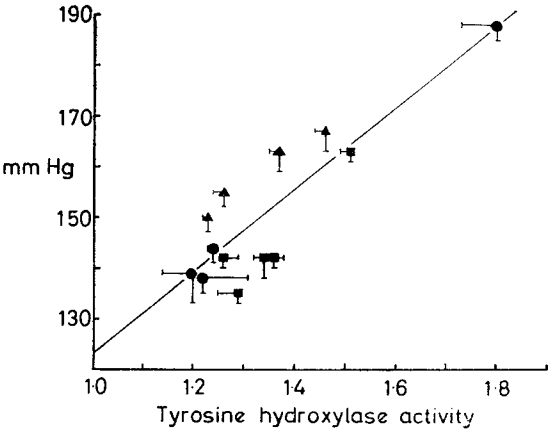

FIG. 3. Correlation between tyrosine hydroxylase activity (mean ± s.e. n = 6) and systolic blood pressure (mm Hg) (mean ± s.e. n = 6) for all groups of rats. Doca/salt—spironolactone (●) angiotensin—saralasin (■) angiotensin—spironolactone (▲). r = 0·874. $P<0·001$ for 11 degrees of freedom.

was not complete until a 20 fold excess of the antagonist was used. That this excess corresponds to the dose used in the clinical antagonism of angiotensin is particularly interesting (Streeten, Anderson & Frieberg, 1975).

Part of the action of angiotensin is to release mineralocorticoid from the zona glomerulosa of the adrenal cortex and therefore the partial blockade of this hypertensive effect of angiotensin by spironolactone shows that there is an indirect contribution to the pressor effect. It can only be assumed that the remainder is due to direct action on the cns. The change in TH activity following the blood pressure response to mineralocorticoid antagonist could have much wider implications. Indeed, the most surprising of all these results is that there is a highly significant degree of correlation between systolic blood pressure and whole brain TH activity for groups of animals receiving all treatments; control, agonist, antagonist and agonist/antagonist in combination.

There must be many possible interpretations of the changes which we have observed in whole brain TH activity in the rat. Alterations in enzyme concentration or activity could be produced in various areas of the brain which would contribute to the overall change. (We have, however, shown that TH activity is increased in both fore and hind brain.)

It has not been possible to demonstrate enzyme induction in these experiments since results following the administration of cycloheximide during the induction of hypertension were difficult to interpret because of the general effect produced on the animals including serious loss of weight.

Nakamura, Gerold & Thoenen (1971), and Chalmers (1975) have suggested that noradrenaline in the brain stem is inhibitory and, using this argument, its regulating enzyme, TH, might well have increased in activity in order to reduce the elevation of the systolic blood pressure. A possible alternative explanation is related to the observation of Finch, Haeusler & Thoenen (1972) that intraventricular 6-hydroxydopamine prevents the induction of doca/salt hypertension. This suggests that the integrity of the central noradrenergic neurons is essential for the development of raised blood pressure.

Our results from angiotensin and mineralocorticoid administration would favour an interpretation in which there is a direct relation between TH activity increase and systolic blood pressure and would suggest that the increase in pressure follows the increase in TH activity. Further elucidation of these factors will come from an analysis of the time course of TH activity increase in relation to the blood pressure rise. This may then more fully explain the role of the cns and particularly TH in the genesis and maintenance of hypertension.

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