# HYPERTENSION, ANTIHYPERTENSIVE DRUGS, AND NOREPINEPHRINE IN BLOOD VESSELS

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IT IS controversial whether hypertension is associated with an abnormal activity of the sympathetic nervous system (DE CHAMPLAIN *et al.*, 1968; DE CHAMPLAIN *et al.*, 1969; DE CHAMPLAIN *et al.*, 1972; HICKLER *et al.*, 1970; LOUIS *et al.*, 1973; LOUIS *et al.*, 1969; MOLINOFF *et al.*, 1972; NAGATSU *et al.*, 1964; SPECTOR *et al.*, 1972; TARVER *et al.*, 1971; YAMORI *et al.*, 1970). One approach to this question has been to utilize animal models of hypertension (DE CHAMPLAIN *et al.*, 1968; DE CHAMPLAIN *et al.*, 1969; DE CHAMPLAIN and VAN AMERINGEN, 1972; LOUIS *et al.*, 1973; LOUIS *et al.*, 1969; OKAMOTO, 1969) and examine the dynamics of catecholamine metabolism in the heart, adrenal glands and brain. Our studies have focused on the disposition of norepinephrine in blood vessels (BERKOWITZ *et al.*, 1972; BERKOWITZ *et al.*, 1971; SPECTOR *et al.*, 1972; TARVER *et al.*, 1971;) and in this review we will summarise our findings in two hypertensive model systems: in the genetic spontaneously hypertensive rat (SHR) (OKAMOTO, 1969) and in the uninephrectomised–deoxcorticosterone acetate (DOCA)-salt treated rat (DE CHAMPLAIN *et al.*, 1968; DE CHAMPLAIN *et al.*, 1969; DE CHAMPLAIN and VAN AMERINGEN, 1972). We also will present studies on the effects of anti-hypertensive drugs on vascular norepinephrine disposition and metabolism in normal and hypertensive animals.

## HYPERTENSION

### *SHR*

It is clear that blood vessels synthesise catecholamines and possess both anabolic and catabolic enzymes (BERKOWITZ *et al.*, 1972; BERKOWITZ *et al.*, 1971; GILLIS and ROTH, 1970; TARVER *et al.*, 1971; TRAJKOV *et al.*, 1973; VERITY *et al.*, 1972). In order to better assess the role of the adrenergic neuro-transmitter in hypertension we have examined in the vasculature those enzymes involved in its metabolism.

The activity of tyrosine hydroxylase in the mesenteric artery was markedly reduced in the SHR compared to normotensive Wistar rats (Fig. 1). We also utilized a "back-crossed SHR" with an intermediate degree of hypertension which was obtained by mating the SHR with normotensive Wistar rats. These rats had an intermediate fall in tyrosine hydroxylase activity in the mesenteric artery. There thus appeared to be an inverse relationship between blood pressure and tyrosine hydroxylase activity.

Aromatic L-amino acid decarbocylase activity may also be decreased in the vasculature of the SHR as Tanaka has reported a decreased activity of this enzyme in cerebral blood vessels (TANAKA, 1972)

Another of the enzymes of catecholamine metabolism that has been the subject of increasing attention, particularly as a possible marker of sympathetic activity, is dopamine-$\beta$-hydroxylase (DBH). Only a few studies pertaining to vascular DBH have been presented. Based on indirect evidence, Weinshilboum and Axelrod (WEINSHILBOUM and AXELROD, 1973) suggested that the bulk of circulating DBH may originate

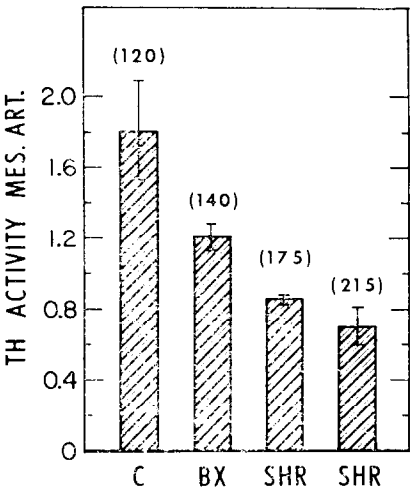


FIG. 1.—Tyrosine hydroxylase (TH) activity of the mesenteric artery in hypertensive rats. C = control normotensive Wistar; BX = Backcrossed SHR-Wistar, SHR = spontaneously hypertensive. Parentheses above bars indicate systolic blood pressure.

from blood vessels. ROFFMAN *et al.* (1973), found a direct relationship between alterations in vascular DBH and circulating DBH and HARTMAN and UDENFRIEND (1972) reported a high concentration of DBH in kidney blood vessels using a histofluorescent method. Utilising a spectrophotometric method (NAGATSU and UDENFRIEND, 1972) we measured the activity of DBH in the mesenteric artery of the SHR and found that the activity of this enzyme was reduced by 50 per cent in these hypertensive rats (TRAJKOV *et al.*, 1973).

The regulation of catecholamine metabolism has been primarily associated with an alteration in synthesis. The role of degradation has been less extensively studied. VERITY, SU and BEVAN (1972) measured catechol-*O*-methyl transferase (COMT) activity in the aorta of normal rabbits and found the highest enzyme activity in the medial layer. In the hypertensive rat, Crevling reported that COMT activity is increased in the heart, liver and kidney CREVLING *et al.*, 1969, but did not examine blood vessels. We have found that the COMT activity is elevated in the aorta (+50%), mesenteric artery (+60%) and heart (+40%) of the SHR with the highest activity seen in those rats having the highest blood pressure. In contrast, monoamine oxidase activity was unaltered in the heart and mesenteric artery of the SHR.

*DOCA-salt-uninephrectomised hypertensive rats*

The interrelationship between hypertension and vascular catecholamines in another model of hypertension, the DOCA-salt-uninephrectomised rat (DE CHAMPLAIN *et al.*, 1968; DE CHAMPLAIN *et al.*, 1969; DE CHAMPLAIN and VAN AMERINGEN, 1972) was examined. Tyrosine hydroxylase activity was altered in a biphasic fashion in this hypertensive specie as shown in Table 1. As hypertension developed, tyrosine hydroxylase was initially increased in the mesenteric artery. However, as the pressure continued to rise, the tyrosine hydroxylase activity began to fall below normal values. Dopamine-$\beta$-hydroxylase activity may also be diminished in the vasculature of these rats. With respect to degradative enzymes, their activity was increased in the

TABLE 1. TYROSINE HYDROXYLASE (TH), MONOAMINE OXIDASE (MAO) AND CATECHOL-*O*-METHYL TRANSFERASE (COMT) ACTIVITY IN THE MESENTERIC ARTERY OF THE DOCA-SALT-UNINEPHRECTOMISED HYPERTENSIVE RAT

| B.P. (mmHg) | Time (days) | TH† | MAO† activity | COMT† |
|---|---|---|---|---|
| 115–120 | 0 | 100 | 100 | 100 |
| 130–135 | 15 | 152* | 95 | — |
| 135–140 | 21 | 130* | — | — |
| 160–170 | 32 | 76* | 169* | — |
| 170–180 | 50 | 77* | — | 150 |
| >210 | 62 | 47* | 176* | — |

* $P < 0{\cdot}05$ compared to control rats.

† Enzyme activities are as percentage of values obtained from control rats with B.P. 115–120 mmHg.

mesenteric artery. Monoamine oxidase and COMT activities rose by 50–70 per cent (Table 1). In DOCA-salt hypertension it is likely that tissue hypertrophy is a factor in the alterations of the metabolic enzymes.

Our findings in both of these models of hypertension show that the activity of synthetic catecholamine enzymes in the mesenteric artery tend to decrease whereas the activity of the degradative enzymes appear to increase. We propose two hypotheses: (1) that both the enzymes of synthesis and degradation may be altered simultaneously to regulate neurotransmitter activity and (2) that in these hypertensive rats, the enzymatic changes represent a compensatory response by the sympathetic nervous system to diminish the vasoconstrictor stimulus of norepinephrine at the vascular level. Although the synthesis of norepinephrine is clearly diminished in the heart of the SHR (LOUIS *et al.*, 1969) it should be recognised that it remains to be established whether the turnover or release of catecholamines by vascular beds *in vivo* are altered by hypertension. Moreover the enzymatic adaptations noted in the aorta and mesenteric arteries of hypertensive rats need not necessarily mean that identical enzymatic alterations occur in veins or in all arterial beds.

## ANTIHYPERTENSIVE DRUGS

Many of the drugs which are used to treat hypertension interfere with the function of the sympathetic nervous system. Perhaps that antihypertensive drug for which there is the best evidence for its mechanism of action involving norepinephrine and the sympathetic nervous system is reserpine. We have previously shown that reserpine depletes vascular and cardiac norepinephrine stores in the normotensive rat and that mesenteric artery tyrosine hydroxylase activity was increased whereas monoamine oxidase activity was decreased (BERKOWITZ *et al.*, 1971). Since hypertension can alter vascular catecholamine enzymes (TARVER *et al.*, 1971) it was not surprising that reserpine-induced hypotension also modified these enzymes. However, recent evidence indicates that alterations in amine concentration may also be a determinant of catecholamine enzyme levels (DAIRMAN, 1972; MOLINOFF, 1972). We therefore administered reserpine and other hypotensive drugs to the SHR in doses which reduced blood pressure towards normal but did not result in a fall in blood pressure below that of a normotensive rat and measured the activity in the mesenteric artery of the synthetic catecholamine enzymes, dopamine-$\beta$-hydroxylase and tyrosine hydroxylase.

Reserpine administration resulted in a fall in systolic blood pressure from 190 mm to 135 mm Hg and a 60 per cent increase in vascular dopamine-$\beta$-hydroxylase activity (Table 2). Moreover tyrosine hydroxylase activity was also increased (C. KOHLER, unpublished observation). When pressure was reduced in the SHR with phenoxybenzamine, an $\alpha$-receptor blocker, dopamine-$\beta$-hydroxylase activity was again increased (Fig. 2).

In order to lower blood pressure without also depleting catecholamines, L-dopa was administered daily for 3 weeks. In contrast to the two previous drugs, no change

TABLE 2. INFLUENCE OF RESERPINE† ON THE DOPAMINE-$\beta$-HYDROXYLASE (DBH) AND TYROSINE HYDROXYLASE (TH) ACTIVITY IN THE MESENTERIC ARTERY OF THE SHR

| Treatment | B.P.‡ | DBH‡ | TH |
|---|---|---|---|
| SHR | 193/148 | 50 ± 0·4 | 0·98 ± 0·08 |
| SHR + Reserpine | 136/100 | 72 ± 0·6* | 2·15 ± 0·20* |

* Significantly differ from control SHR, $P < 0{\cdot}05$.

† Reserpine was given in a dose of 0·25 mg/kg i.p. once a day for 4 days and rats killed on day 5.

‡ DBH activity is as nmol octopamine/hr/mg protein. The results are the average ± standard error of the mean from 8–13 animals for each group. Blood pressures are the average systolic/diastolic pressure. Tyrosine hydroxylase activity is expressed as nmoles Dopa/15 min/mg/protein.

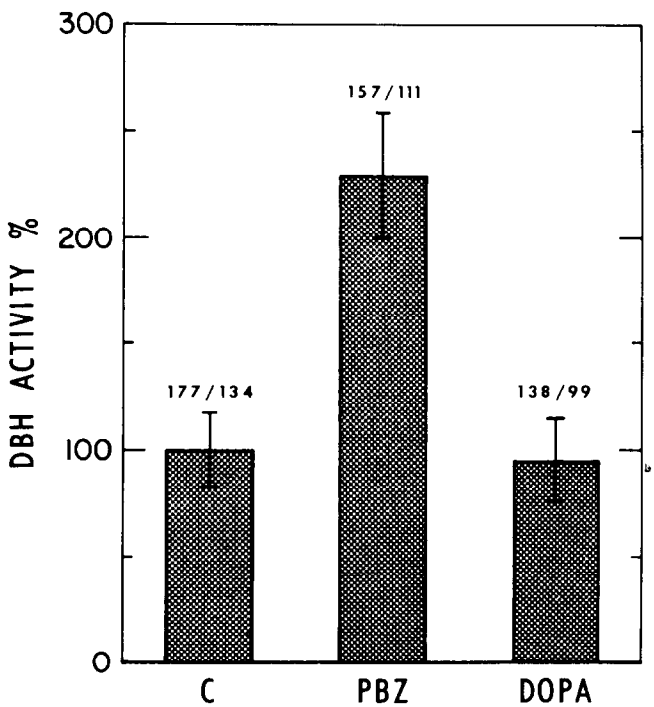


FIG. 2.—Effect of reduction of blood pressure by phenoxybenzamine or L-dopa on the dopamine-$\beta$-hydroxylase (DBH) activity in the mesenteric artery of the SHR. Results are expressed as percentage control DBH activity. Phenoxybenzamine was given in a dose of 10 mg/kg once a day for 2 days and rats killed on the third day. L-dopa was given once daily s.c. for 3 weeks, 300 mg/kg, week one, 600 mg/kg, week two and 1000 mg/kg, week three. Systolic/diastolic blood pressure is shown above bars. Results are the average of 5–8 determinations.

in dopamine-$\beta$-hydroxylase accompanied the fall in blood pressure (Fig. 2). Similar results have been obtained utilizing the monoamine oxidase inhibitor, pargyline (unpublished observation). It appears that both blood pressure and amine concentration are important in determining whether vascular catecholamine enzyme activity is altered. Moreover, a drug which lowers blood pressure but increases the activity of catecholamine synthetic enzymes in blood vessels may initiate mechanism which tend to limit its action or result in tolerance to its antihypertensive effects.

TABLE 3. FEEDBACK CONTROL OF NOREPINEPHRINE SYNTHESIS

| Tissue (N)† | Drug | Time | $\mu$g/g (%$\Delta$) | NE† dis/min per g (%$\Delta$) | NE† dis/min per $\mu$g (%$\Delta$) |
|---|---|---|---|---|---|
| Heart (8) | Control | — | 1·83 ± 0·08 | 2233 ± 321 | 1205 ± 392 |
| Heart (8) | MAOI | 24 | 3·18 ± 0·01 (+73) | 699 ± 137*(−68) | 214 ± 38* (−82) |
| Aorta (3) | Control | — | 1·66 ± 0·71 | 649 ± 80 | 488 ± 122 |
| Aorta (3) | MAOI | 24 | 1·55 ± 0·32 (−7) | 399 ± 58* (−39) | 273 ± 53 (−44) |
| Mesenteric Art. (3) | Control | — | 2·44 ± 0·16 | 2971 ± 390 | 1205 ± 88 |
| Mesenteric Art. (3) | MAOI | 24 | 6·20 ± 0·30*(+154) | 3233 ± 524 (+9) | 533 ± 118*(−56) |
| Mesenteric Vein (2) | Control | — | 1·03–1·47 | 641–793 | 539–622 |
| Mesenteric Vein (2) | MAOI | 24 | 2·43–3·14 (+122) | 320–481 (−44) | 102–197 (−74) |

Effect of monoamine oxidase inhibition on the concentration and synthesis of $^{14}$C-norepinephrine in the guinea pig cardiovascular system. Pargyline hydrochloride 75 mg/kg was administered i.p. 25 hr prior to sacrifice; 30 $\mu$Ci of $^{14}$C-tyrosine was administered i.v. 1 hr prior to sacrifice.

* Statistically significant $P < 0{\cdot}05$ compared to control tissue.

† (N) indicates the number of individual hearts or groups of vessels assayed. Each group of vessels was pooled from 4 to 5 animals for aorta and mesenteric arteries and the range from two groups of 8 mesenteric veins.

‡ Norepinephrine is dis/min per g tissue or $\mu$g norepinephrine ± S.E.M., (%$\Delta$) indicates percentage difference from control tissues.

Among the most enigmatic of the antihypertensive drugs, are those classed as monoamine oxidase inhibitors. The paradox as to why drugs which can increase the tissue content of norepinephrine, a vasoconstrictor, may also lower blood pressure has not been resolved (NICKERSON, 1970). Drugs which elevate norepinephrine levels could diminish catecholamine synthesis in the vasculature by feedback inhibition (NAGATSU *et al.*, 1964; WEINER *et al.*, 1972) and conceivably diminish the amount of amine reaching the medial smooth muscle. We have studied the end product control of norepinephrine formation in blood vessels and heart of the guinea pig *in vitro* and *in vivo*.

As shown in Table 2, the susceptibility to decreased norepinephrine synthesis after pargyline was heart > mesenteric vein > aorta > mesenteric artery. A 73 per cent elevation in heart norepinephrine content caused an 82 per cent fall in cardiac norepinephrine specific activity (dis/min per $\mu$g) whereas a 154 per cent increase in mesenteric arterial norepinephrine levels, twice that of the heart, resulted in only a 56 per cent decline in norepinephrine specific activity in the artery.

Another index of norepinephrine synthesis is the amount of radioactive norepinephrine formed from tyrosine-$^{14}$C per amount of tissue (dis/min per g tissue). Using this measure of conversion the heart catecholamine synthesis was reduced to a far greater extent than any vascular bed with no reduction in labelled norepinephrine detected in the mesenteric artery.

Because vascular tyrosine hydroxylase is susceptible to catecholamine inhibition *in*

*vitro* we believe it is likely that this mechanism is also operative *in vivo*. However, in some vascular beds such as the mesenteric arteries the high tyrosine hydroxylase levels (SPECTOR *et al.*, 1972; TARVER *et al.*, 1971) may require a greater concentration of catecholamines for further inhibition of the enzyme than can easily be achieved *in vivo*. Therefore after a drug, like a monoamine oxidase inhibitor, end product regulation of norepinephrine synthesis may vary in different parts of the cardiovascular system. The present findings suggest that the heart and veins may be more readily subject to decreases in norepinephrine synthesis by feed-back inhibition than are the arteries. If applicable to man this data may explain the clinical evidence for monoamine oxidase inhibitors decreasing cardiac output and producing postural hypotension but only minimally altering recumbent blood pressure (NICKERSON *et al.*, 1970).

## CONCLUSIONS

(1) In the mesenteric arteries and the aorta of hypertensive rats the enzymes of norepinephrine metabolism are altered. Synthetic enzyme activities are reduced and degradative enzyme activities are increased. We believe that this is a compensatory adaptation to hypertension at the vascular level.

(2) Some anti-hypertensive drugs increased the activity of vascular tyrosine hydroxylase and dopamine-$\beta$-hydroxylase as they reduced blood pressure in the SHR whereas other drugs lowered blood pressure without altering enzyme activities. Both blood pressure and catecholamine concentrations are apparently regulators of enzyme levels in blood vessels.

(3) Monoamine oxidase inhibitors raised the content of norepinephrine in the heart and blood vessels of guinea pigs and diminished norepinephrine synthesis by end product inhibition at the tyrosine hydroxylase step. However, some arterial beds were more resistant to end product regulation of norepinephrine than were the veins or heart.

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