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Hypertension: its effect on the stimulated release of dopamine-β-hydroxylase in the rat

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Plasma dopamine-β-hydroxylase (DBH) concentrations have been postulated as providing an index of sympathetic nerve activity. Using a microspectrophotometric assay system, plasma DBH concentrations have been measured in emergent blood from autoperfused heart, spleen and mesentery of normotensive, deoxycorticosterone (doca)/NaCl-treated, Goldblatt (1 kidney) renal and spontaneously hypertensive rats following sympathetic nerve outflow stimulation. Changes in plasma DBH concentrations as a result of sympathetic nerve outflow stimulation rates of 1–25 Hz for the mesentery and spleen and 1–4 Hz for the heart, were found to be frequency-dependent in all groups. Significantly greater amounts of DBH were found in the perfusate from the spleen (1–25 Hz) and mesentery (3–25 Hz) but not the heart (0·5–4 Hz) of renal hypertensive rats compared with normotensive controls. Significantly greater concentrations of DBH were released from the spleen but not the mesentery in all hypertensive groups following high stimulation frequencies of 12 and 25 Hz. It is concluded that there is a relation between plasma DBH concentrations and sympathetic nerve activity. Furthermore, greater amounts of the enzyme are released from the spleen and mesentery of chronic renal hypertensive rats following sympathetic nerve stimulation.

Increasing evidence suggests that enhanced activity of the peripheral sympathetic nervous system contributes to the development and maintenance of increased arterial blood pressure observed in some of the rat hypertensive model systems doca/NaCl-treated hypertensive rats (de Champlain & Van Ameringen, 1972), renal hypertensive rats (Henning, 1969), spontaneously hypertensive rats (Yamori, Ooshima & Okamoto, 1973).

Dopamine-β-hydroxylase (DBH) is the mixed function oxidase that catalyses the final stage of noradrenaline biosynthesis. The enzyme has been shown to occur within the intracellular vesicles of adrenergic neurons (Geffen & Livett, 1971) and the release of the transmitter during peripheral adrenergic nerve stimulation or spontaneous activity is accompanied by a proportional release of DBH and other constituents of the vesicle (Axelrod, 1972). It has been proposed, therefore, that plasma DBH concentration can directly reflect the amount of neurotransmitter released and thus provide an index of sympathetic neuronal activity (Weinshilboum, Kvetnansky & others, 1971a; Planz & Palm, 1972; Wooten & Cardon, 1973; Freedman, Ebstein & others, 1973; Frewin, Downey & Livitt, 1973). Trajkov, Berkowitz & Spector (1974) showed that in certain types of experimental hypertension, neuronal DBH concentrations were reduced, from which a regulatory function for the enzyme in modulating neurotransmitter outflow was inferred. In the present study we have examined the DBH concentrations occurring in the perfusates from the spleen, mesentery and heart following sympathetic nerve stimulation and compared the effects seen in doca/NaCl-treated, 'Goldblatt' renal and spontaneously hypertensive rats with the responses obtained in normotensive animals. Part of the contents of this paper were communicated to the British Pharmacological Society (Algate & Leach, 1976).

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MATERIALS AND METHODS

Experiments were performed on female normotensive (NT) and experimental hypertensive rats (200–240 g, Bradford University bred—CFE derived) and also on age-matched spontaneously hypertensive rats (SHR, Bradford University bred—Okamoto derived). Surgical procedures to induce hypertension were performed on 6–7 week old rats. The existence of hypertension was defined as a diastolic b.p. $\geqslant$ 160 mmHg in the ether-anaesthetized rat.

Experimental models of hypertension

In addition to spontaneously hypertensive animals, two other experimental models were used.

(i) *Deoxycorticosterone acetate/NaCl.* Doca/NaCl hypertension (DH) (Selye, Hall & Rowley, 1943) was induced by subcutaneous implantation of doca acetate pellets (2 × 25 mg) during halothane-

induced anaesthesia. In one group of rats unilateral nephrectomy was also performed (this group is referred to as DNH). Drinking water was replaced with 0·9% w/v NaCl solution for 4 weeks.

(ii) *Renal arterial clip (RH).* Stenosis of the renal artery was produced by the application of a clip (internal gap 0·25 mm) under halothane anaesthesia, to the left renal artery and removal of the contralateral kidney (Goldblatt, Lynch & others, 1934).

Experimental procedure. Six weeks after surgical procedures the rats were anaesthetized with ether, pithed (Gillespie & Muir, 1967) and artificially respired at 70 strokes min^{-1}, 1 cm^3 per 100 g by means of a SRI ventilator. The left femoral vein and common carotid artery were cannulated with pp25 polythene cannulae and the arterial cannula connected to a Bell and Howell P23 pressure transducer to record blood pressure on a Devices M2 recorder. Tubocurarine (1 mg kg^{-1}, i.v.) was administered before commencement of electrical stimulation. The pithing rod constituted the active electrode for nerve stimulation whilst a flattened serum needle inserted in the dorsal muscles provided the indifferent electrode. Square-wave electrical stimulation was provided by a Multitone stimulator. At appropriate times, 300 μl samples of blood were taken simultaneously from the efferent organ blood supply under investigation and the systemic circulation, and replaced with an equal volume of saline (0·9% w/v). The preparation and assay of plasma DBH has previously been described (Algate & Leach, 1978). The method uses a sensitive dual-wavelength spectrophotometric technique based upon the method of Nagatsu & Udenfriend (1972) as described by Kato, Kuzuya & Nagatsu (1974).

To determine the absolute plasma DBH concentration in the blood flow from the organ under examination, the concentration in the systemic circulation was subtracted from the concentration present in the organ effluent. Thus, for the three organs studied, the total concentration was determined by measurement of DBH plasma concentrations as summarized below.

Spleen/mesentery: DBH concn (organ perfusate) − DBH concn (arterial systemic sample)

Heart: DBH concn (aortic sample) − DBH concn (venous systemic sample).

Preparation of autoperfused organs

(i) *Mesentery.* After cannulation of the femoral vein and common carotid artery, the hepatic portal vein was cannulated using heparinized saline-filled pp25 polythene tubing and connected to the femoral vein cannula for the maintenance of blood flow. Stimulation of the mesentery was achieved through a varnished pithing rod with 3·5 cm of exposed metal at its tip, which was positioned in the general area of the mesenteric-coeliac sympathetic outflow (T10-L1). The sympathetic nerve outflows to the mesentery were stimulated for 5 s (20 V, 0·3 ms) at 1, 3, 6, 12, and 25 Hz. Blood samples were collected 2 min after stimulation began. Thirty min recovery time was allowed between each period of stimulation.

(ii) *Spleen.* After the initial cannulation as described earlier, the splenic vein was cannulated using heparinized saline-filled pp25 polythene tubing and connected to the femoral vein cannula to maintain the circulation. The experimental design for selective sympathetic splenic nerve stimulation was similar to that used for the mesentery, except that samples were collected 3 min after application of the stimulus.

(iii) *Heart.* A modified pithing rod (Algate & Leach, 1978) was used in these experiments for the selective stimulation of cardiac sympathetic nerves. Positioning of the electrodes at the spinal outflow to the heart (approximately C7-T1) was achieved by applying a continuous square-wave stimulus (25 V, 0·5 ms, 4 Hz) and withdrawing the electrode rod until a maximum tachycardia was produced. Plasma samples were removed from the aortic arch via a retrograde cannula inserted through the common carotid artery. The cannula was flushed with 0·25 ml heparinized saline and then blood was allowed to flow into the collecting tube. A control sample was withdrawn simultaneously from the inferior vena cava via the femoral vein cannula. Blood samples were collected 1 min after 15 s stimulation (0·5 ms, 30 V) at frequencies of 0·5, 1, 2 and 4 Hz.

Statistical Analysis

Student's *t*-test was used for evaluation of data. Group mean values are quoted together with the standard error of the means (s.e.).

RESULTS

Spleen. Increases in the stimulation frequency (1–25 Hz) of the sympathetic nerve outflow to the spleen evoked a proportional change in the DBH concentration in the plasma perfusate of normotensive animals measured at the time of stimulation. The stimulation frequency—DBH concentration relation was also determined for separate groups of SHR, DH, DNH and RH animals and the results are summarized in Fig. 1.

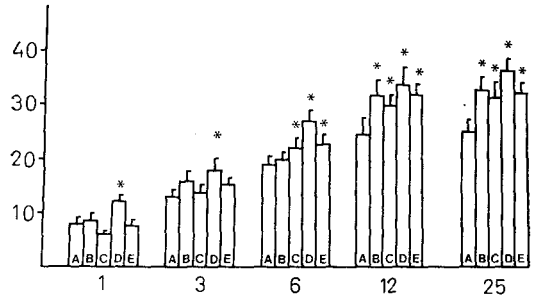

FIG. 1. The effects of sympathetic nerve outflow stimulation frequency on the plasma DBH concentration measured in the perfusate from the splenic vein. Blood was sampled 3 min following electrical stimulation (20 V, 0·3 ms, 5 s) of the spinal cord (T10–L1). Ordinate: the difference in plasma DBH concentrations (n mol cm^{-3} h^{-1}) between the splenic venous and common carotid systemic samples, this indicates the enzyme concentration released by the spleen. Abscissa: sympathetic outflow stimulation frequency (Hz) in normotensive and various types of hypertensive rats. A—Normotensive control; B—doca hypertensive; C—doca/unilateral nephrectomy hypertensive; D—Renal hypertensive; E—Spontaneous hypertensive; *$P \leqslant 0{\cdot}01$. n = 8.

Significantly greater amounts of DBH were seen to be present in the splenic plasma perfusate flow of the RH group over the entire stimulation frequency range (1–25 Hz) compared with the corresponding frequency response in NT rats. In the DH, DNH and SHR groups the responses to stimulation at low frequencies (1 and 3 Hz) were not significantly different from the NT controls. However, at 6, 12 and 25 Hz, the DBH release was significantly greater than that released in control rats (Table 1).

Mesentery. Changes in the stimulation frequency of the sympathetic nerve outflow (1–25 Hz) to the mesenteric vascular region evoked a proportional change in the plasma DBH perfusate concentration collected from the hepatic portal vein in normotensive rats. A frequency related increase in DBH activity in the venous perfusate was also observed in all four hypertensive groups examined (Fig. 2).

Significantly greater changes in DBH concentrations following stimulation at 3, 6, and 12 Hz occurred in the RH group compared with the NT controls. The changes in DBH concentration following stimulation in DH, DNH and SHR groups were not significantly different from those observed in the NT group (Table 2).

Heart. In the NT, DNH, RH and SHR groups, electrical stimulation of the sympathetic outflow to the stellate ganglion at 0·5 Hz induced an overall decrease in the DBH concentration in plasma perfusing the heart at the time of stimulation (Table 3). An insignificant increase in DBH concentration was only observed in the DH group. Higher stimulation frequencies (1, 2 and 4 Hz) evoked a frequency related increase in DBH concentrations. The

Table 1. *The plasma DBH concentration was measured in 300 μl blood samples removed from the splenic vein and common carotid artery.* Blood was sampled 3 min following square wave sympathetic nerve outflow stimulation (20 V, 0·3 ms, 5 s) of the spinal cord (T10–L1). The values shown in the table represent the difference in DBH concentration of the two samples and indicate the increased enzyme concentration released from the spleen. A comparison is made between the response to nerve stimulation at various electrical frequencies in normotensive and various types of hypertensive rats. n = 8.

Condition	Plasma DBH concn (nmol cm^{-3} h^{-1}) at stimulation frequencies (Hz) of: 1	3	6	12	25
NT	8·1 (1·3)	13·1 (1·5)	18·4 (1·4)	24·7 (3·1)	25·3 (2·3)
DH	8·8 (1·3)	15·9 (1·8)	19·9 (1·3)	31·9* (2·6)	33·3* (2·4)
DNH	6·3 (0·6)	13·8 (1·6)	22·2* (1·4)	30·1* (1·9)	31·8* (2·9)
RH	12·1* (0·9)	18·1* (1·9)	26·9* (2·3)	34·1* (3·2)	36·9* (2·3)
SHR	7·8 (1·0)	15·3 (1·3)	22·8* (1·8)	32·2* (1·9)	32·7* (1·9)

NT normotension control; DH doca; DNH doca/unilateral nephrectomy; RH renal; * $P \leqslant 0{\cdot}01$.

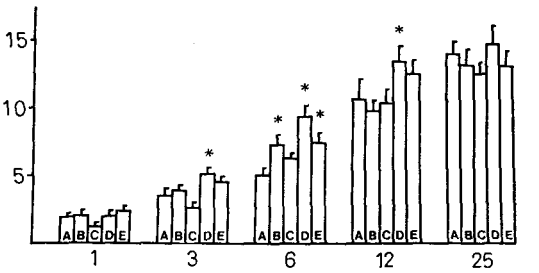

FIG. 2. The effects of sympathetic nerve outflow stimulation frequency on the plasma DBH concentration measured in the perfusate from the hepatic portal vein. Blood was sampled 2 min following electrical stimulation (20 V, 0·3 ms, 5 s) of the spinal cord (T10–L1). Ordinate: the difference in plasma DBH concentrations (n mol cm^{-3} h^{-1}) between hepatic portal venous and common carotid systemic samples 1, this indicates the enzyme concentration released by the mesentery. Abscissa: sympathetic outflow stimulation frequency (Hz) in normotensive and various types of hypertensive rats. A—Normotensive control; B—doca hypertensive; C—doca/unilateral nephrectomy hypertensive; D—Renal hypertensive; E—Spontaneous hypertensive. * $P \leqslant 0{\cdot}01$. n = 8.

increased concentration of plasma DBH and its relation to sympathetic stimulation frequency in all four hypertensive groups, were not significantly different from the changes occurring in the NT controls.

DISCUSSION

We previously showed that the DBH concentration in plasma effluxing from the heart, spleen and mesentery was significantly increased from resting concentrations following stimulation of the sympathetic nerve outflow to these organs (Algate & Leach, 1978). The present results show that a positive relation exists between the plasma DBH concentration in the venous effluent from the spleen and mesentery and the frequency of sympathetic nerve stimulation (1–25 Hz). This frequency-relation can be clearly seen in the normotensive as well as all four hypertensive models. A similar correlation between the plasma DBH concentration in aortic serum samples and the frequency of cardiac sympathetic nerve stimulation was also observed at 1, 2 and 4 Hz. At the slowest stimulation frequency (0·5 Hz) a decrease in plasma DBH concentration was seen in the heart of all groups except DH. Such decreases probably occur as the result of increases in cardiac output, arising from sympathetic stimulation increased blood flow through the heart and lungs, which would result in a more rapid dilution of the enzyme concentration. This factor would still exist at the higher frequencies used in the experiment, but because the DBH concentration is increased the dilution factor would become less important. These results are considered to be in general agreement with the hypothesis that the plasma DBH originates from sympathetic neurons during the periods of nerve stimulation and that the DBH concentration is proportional to the frequency of nerve stimulation (Weinshilboum, Thoa & others, 1971b). Differences in the concentration of DBH released from the organs of hypertensive rats were only observed in the spleen and mesentery. Significantly greater amounts of DBH were found in the venous effluent from the spleen and mesentery of renal hypertensive rats over a stimulation frequency range of 3-12 Hz. This finding may reflect facilitated release of the enzyme from the neuron or an increase in the soluble fraction of the enzyme present in the terminal vesicles and could be interpreted as increased noradrenaline transmitter release in this model. Because the noradrenaline release was not measured simultaneously, these results do not allow for more precise conclusions. Chevillard, Duchene & Alexandre (1975) showed that elevated serum

Table 2. *The plasma DBH concentration was measured in 300 μl blood samples removed from the hepatic portal vein and common carotid artery.* Blood was sampled 2 min following square wave sympathetic nerve outflow stimulation (20 V, 0·3 ms, 5 s) of the spinal cord (T10–L1). The values shown in the Table represent the difference in DBH concentration of the two samples and indicate the increased enzyme concentrations released from the mesentery. A comparison is made between the response to nerve stimulation at various electrical frequencies in normotensive and various types of hypertensive rat. n = 8.

Condition†	Plasma DBH concn (nmol cm^{-3} h^{-1}) at stimulation frequencies (Hz) of: 1	3	6	12	25
NT	2·00 (0·26)	3·60 (0·49)	5·08 (0·52)	10·8 (1·44)	14·26 (0·92)
DH	2·14 (0·33)	3·95 (0·31)	7·36* (0·68)	9·93 (0·73)	13·40 (1·22)
DNH	1·29 (0·28)	2·73 (0·26)	6·31 (0·42)	10·47 (0·94)	12·68 (1·11)
RH	2·09 (0·26)	5·20* (0·38)	9·46* (0·82)	13·65* (1·20)	15·06 (1·39)
SHR	2·47 (0·26)	4·59 (0·35)	7·48 (0·68)	12·71 (10·6)	13·41 (1·18)

* $P \geqslant 0.05$.
† See Table 1.

Table 3. *The plasma DBH concentration was measured in 300 μl blood samples removed from the aortic arch and inferior vena cava.* Blood was sampled 1 min following square wave sympathetic nerve outflow stimulation (25 V, 0·5 ms, 15 s) of the spinal cord (C7–T1). The values shown in the table represent the difference in DBH concentration of the two samples and indicate the increased enzyme concentration released from the heart. A comparison is made between the response to nerve stimulation at various electrical frequencies in normotensive and various types of hypertensive rats. n = 8.

Condition†	Plasma DBH concn (nmol cm^{-3} h^{-1}) at stimulation frequencies (Hz) of: 0·5	1	2	4
NT	−1·49 (0·61)	2·40 (0·54)	5·57 (1·22)	9·13 (1·69)
DH	+0·86 (1·20)	3·21 (1·10)	4·73 (0·98)	7·30 (1·03)
DNH	−0·96 (0·39)	1·64 (0·86)	5·36 (1·49)	11·30 (1·57)
RH	−1·84 (0·98)	1·69 (1·18)	4·85 (1·13)	8·38 (0·78)
SHR	−0·49 (0·81)	2·28 (0·51)	4·85 (0·54)	8·40 (0·96)

† See Table 1.

angiotensin II concentrations, a feature accompanying experimental renal hypertension (Bumpus, Sen & others, 1973; Miksche, Miksche & Gross, 1970; Brunner, 1975) can increase the noradrenaline concentration present in cardiac tissue slices. An increase in the concentration of DBH present was postulated as the reason since the kinetics of β-hydroxylation were unchanged. Hughes & Roth (1971) demonstrated augmented noradrenaline release from the rabbit coeliac artery in the presence of angiotensin II, following electrical stimulation *in vitro*. Applying the hypothesis that DBH is released with noradrenaline from adrenergic sympathetic nerves (Weinshilboum & others, 1971b), augmented noradrenaline release would be associated with augmented DBH release in the presence of angiotensin II. Evidence not in support of our postulation is provided by Finch & Leach (1969), who showed that relatively high concentrations of angiotensin II are necessary before the indirect release of noradrenaline occurs. DBH estimations were not made in their experiments, but proportional and concomitant changes in the enzyme may be inferred if the theory of coupled release is correct. Clearly, further experiments are required to elucidate this phenomenon.

The finding of significantly greater concentrations of DBH released from the spleen in all four hypertensive models compared with normotensive animals at the higher stimulation frequencies (12 and 25 Hz) cannot easily be explained. These frequencies are probably beyond the range considered 'normal' for sympathetic nerve activity (Iriuchijima, 1973) and since the corresponding effect was not observed in the mesentery, the importance of the results must be viewed with caution.

Nevertheless, the ability to measure plasma DBH concentrations does offer a means for investigating further the relation of the sympathetic nervous system to the development of experimental hypertension.

The clearly significant findings seen with renal hypertensive rats, compared with the smaller changes in enzyme concentration which occurred with the spontaneously and doca/NaCl hypertensive models, support the view that the models achieve their elevated blood pressure by different mechanisms.

REFERENCES

ALGATE, D. R. & LEACH, G. D. H. (1976). *Br. J. Pharmac.*, **58**, 275P.

ALGATE, D. R. & LEACH, G. D. H. (1978). *J. Pharm. Pharmac.*, **30**, 162–166.

AXELROD, J. (1972). *Pharmac. Rev.*, **24**, 233–243.

BRUNNER, H. R. (1975). In: *Pathophysiology and management of arterial hypertension.* Editors: Berglund, G., Hansson, L. & Werko, L., Lindgren and Soner, Molndal, Sweden.

BUMPUS, F. M., SEN, S., SMEBY, R. R., SWEET, C., FERRARIO, C. M. & KHOSLA, M. C. (1973). *Circulation Res.*, **32/33**, *Suppl.*, **1**, 150–158.

DE CHAMPLAIN, J. & VAN AMERINGEN, M. R. (1972). *Ibid.*, **31**, 617–628.

CHEVILLARD, C., DUCHENE, N. & ALEXANDRE, J. M. (1975). *J. Pharm. Pharmac.*, **27**, 193–196.

FINCH, L. & LEACH, G. D. H. (1969). *Br. J. Pharmac.*, **36**, 481–488.

FREEDMAN, L. S., EBSTEIN, R. P., PARK, D. H., LEVITZ, S. M., GOLDSTEIN, M., DAVIS, S., CHU, D. S. & MANGER, W. M. (1973). *Res. Commun. Chem. Path. Pharmac.*, **6**, 873–878.

FREWIN, D. B., DOWNEY, J. A. & LIVITT, M. (1973). *J. Physiol. Pharmac.*, **51**, 986–989.

GEFFEN, L. B. & LIVETT, B. G. (1971). *Physiol. Rev.*, **51**, 98.

GILLESPIE, J. S. & MUIR, T. C. (1967). *Br. J. Pharmac.*, **30**, 78–87.

GOLDBLATT, H., LYNCH, J., HANZAL, R. F. & SUMMERVILLE, W. W. (1934). *J. exp. Med.*, **59**, 347–379.

HENNING, M. (1969). *J. Pharm. Pharmac.*, **21**, 61–63.

HUGHES, J. & ROTH, R. H. (1971). *Br. J. Pharmac.*, **41**, 239–255.

KATO, J., KUZUYA, H. & NAGATSU, T. (1974). *Biochem. Med.*, **10**, 320–328.

IRIUCHIJIMA, J. (1973). *Jap. Heart J.*, **14**, 350–356.

MIKSCHE, L. W., MIKSCHE, U. & GROSS, F. (1970). *Circulation Res.*, **27**, 973–984.

NAGATSU, T. & UDENFRIEND, S. (1972). *Clin. Chem.*, **18**, 980–983.

PLANZ, G. & PALM, D. (1972). *Eur. J. clin. Pharmac.*, **5**, 255–258.

SELYE, H., HALL, C. E. & ROWLEY, E. M. (1943). *Can. med. Ass. J.*, **49**, 88–92.

TRAJKOV, T., BERKOWITZ, B. A. & SPECTOR, S. (1974). *Blood Vessels*, **11**, 101–109.

WEINSHILBOUM, R. M., KVETNANSKY, R., AXELROD, J. & KOPIN, I. J. (1971a). *Nature, New Biol.*, **230**, 287–288.

WEINSHILBOUM, R. M., THOA, N. B., JOHNSON, D. G., KOPIN, I. J. & AXELROD, J. (1971b). *Science*, **174**, 1349–1351.

WOOTEN, G. F. & CARDON, P. V. (1973). *Arch. Neurol.*, **28**, 103–105.

YAMORI, Y., OOSHIMA, A. & OKAMOTO, K. (1973). *Jap. Circ. J.*, **37**, 1235–1245.