J. Pharm. Pharmac., 1978, 30, 162–166 Received July 28, 1977

Dopamine-β-hydroxylase release following acute selective sympathetic nerve stimulation of the heart, spleen and mesentery

D. R. ALGATE* AND G. D. H. LEACH

School of Pharmacology, University of Bradford, Bradford, West Yorkshire, U.K.

An increase in the concentration of dopamine-β-hydroxylase (DBH) in the blood perfusates of the heart, spleen and mesentery of pithed rats was detected following selective sympathetic nerve stimulation. Furthermore, the concentration of enzyme released by each organ correlated with the stimulation frequency. The results provide further corroborative evidence that the concentration of DBH in the plasma is related to the degree of sympathetic tone. If the sampling methods described were applied to non-spinalized animals, the concentrations of DBH in organ perfusates could be used as an index of sympathetic nervous tone and thus provide a useful preparation for the study of drugs on the sympathetic nervous system.

Dopamine-β-hydroxylase (DBH) is the mixed function oxidase that catalyses the synthesis of noradrenaline from dopamine in the neuronal vesicles of adrenergic nerves. Its release is thought to accompany that of noradrenaline following nerve stimulation (Duch, Viveros & Kirshner, 1968; Geffen & Livett, 1971). It has been suggested therefore, that the concentration of circulating serum DBH could provide a useful index of sympathetic nervous activity (Axelrod, 1972). Although raised DBH concentrations have been observed following acute stress in man (Wooten & Cardon, 1973) and rats (Weinshilboum, Kvetnansky & others, 1971 b), no clear relation has yet been established between the concentration of DBH in the blood and sympathetic nerve activity in situations involving chronic changes in nervous tone. One possible reason for the lack of correlation is the extreme variability in base-line serum DBH concentrations between individuals (Wooten & Ciaranello, 1974). It has been suggested that this variability is due to the presence of both active and inactive forms of the enzyme (Rush & Geffen, 1972; De Quattro & Miura, 1973). The concentration of DBH in the circulation is a sum of the enzyme released from all sympathetic nerve endings and not just those involved in cardiovascular control; in addition, the enzyme is released from the adrenal glands. Furthermore, the proportion of the total DBH concentration released into the systemic circulation is likely to be different for each tissue. It is our opinion, therefore, that changes in the circulation plasma DBH concentration do not accurately reflect changes in the sympathetic nervous system. If a conclusive relation is to be established between the serum concentration of DBH and the state of sympathetic nerves, enzyme measurements should be made close to the point of its release. We have therefore examined the concentration of DBH present in the blood perfusates of the spleen, mesentery and heart following selective sympathetic nerve stimulation of the relevant preganglionic sympathetic nerves at various frequencies.

* Correspondence and present address: Wyeth Institute of Medical Research, Huntercombe Lane South, Taplow.

METHODS

Experiments were performed on female rats (C.F.E. derived, 200–240 g) anaesthetized with ether. The animals were pithed by the method of Gillespie & Muir (1967) and artificially ventilated at 70 strokes min^{-1}, with a stroke volume of 1 ml/100 g. The left femoral vein and left carotid artery were cannulated and the latter connected to a pressure transducer and pen recorder. Tubocurarine (1 mg kg^{-1}, i.v.) was administered before any electrical stimulation was applied.

The pithing rod acted as the active electrode for nerve stimulation whilst a flattened serum needle inserted in the dorsal muscles formed the indifferent electrode. Electrical stimulation was provided by a square-wave multitone stimulator. At appropriate times a 300 μl sample of blood was taken simultaneously from the organ under investigation and the systemic circulation. Following sample removal, an equal volume of saline was intravenously injected to restore the circulatory volume. Blood samples were centrifuged at 4500 rev min^{-1} and the DBH activity determined by sensitive dual-wavelength spectrophotometry based on the spectrophotometric method

of Nagatsu & Udenfriend (1972) as described by Kato, Kuzuya & Nagatsu, (1974). The incubation mixture (total volume 1 ml) contained 400 μl of 4-fold diluted serum, 200 μmol of sodium acetate buffer, pH 5·0, 10 μmol of sodium fumarate, 10 μmol of ascorbic acid, 1 μmol of pargyline, 1500 units of catalase, 20 μmol of tyramine (substrate), and for inactivation of endogenous inhibitors 10 μmol of *N*-ethylmaleimide and 0·5 nmol of $CuSO_4$ was included. Incubation was at 37° for 60 min. A sample of diluted plasma, heated to 95° for 5 min, was taken through all of the stages to serve as a blank incubation. The reaction was stopped by the addition of 3M trichloroacetic acid (0·2 ml) and the amines in the supernatant were isolated on a Dowex 50W–X4 microcolumn and eluted with NH_4OH. The formed octopamine in the eluate was converted to *p*-hydroxybenzaldehyde with 2% $NaIO_4$ (10 μl) and extracted with ether and NH_4OH. The difference in absorbance of samples at 330 and 360 nm was measured with a spectrophotometer. Boiled plasma control samples gave low values of absorption, the blanks being recorded in the range 0·012–0·015, and an internal standard containing 2 nm of octopamine was run with each batch. The absorbance of these standard samples was 2–3 times that of the blank reading. DBH activity is expressed as nanamoles of octopamine formed per hour per ml of plasma.

Mesentery

The hepatic portal vein was connected to a polythene cannula introduced into the femoral vein to maintain the circulation of blood through the mesentery. 30 min was then allowed for the preparation to stabilize. Selective stimulation of the lower sympathetic outflow was achieved with a varnished pithing rod with 3·5 cm of exposed metal at the tip. The exposed length of rod was positioned in the general area of the mesenteric-coeliac outflow (T10-L1) by pushing it fully down the spine and then withdrawing it 1–2 cm.

Time course of DBH release

The sympathetic outflow to the mesentery was stimulated for 5 s (20 V, 0·3 ms, 12 Hz). This voltage was chosen since higher values produced marked changes in heart rate, indicating that the stimulus had spread to nerves higher up the spinal cord. Samples of mesenteric perfusate were obtained by opening the tubing links between the hepatic portal and femoral vein and allowing the blood to collect in a polythene sample-tube. A control arterial sample was collected simultaneously from the carotid artery in a similar manner. Samples were removed 1, 2, 4, 8 and 16 min following stimulation.

Frequency responses

The sympathetic nerves to the mesentery were stimulated for 5 s (20 V, 0·3 ms) at frequencies of 1, 3, 6, 12 and 25 Hz. Blood samples were collected 2 min after the stimulation commenced. 30 min were allowed for recovery after each period of stimulation.

Spleen

The splenic vein was cannulated and connected to the femoral vein cannula to maintain circulation. To study the time course of DBH release, samples were collected 3 min after the application of the stimulus from the splenic vein and carotid artery. Stimulation frequencies and collection times were similar to that described for the mesentery.

Heart

A modified pithing rod was used. The rod bore two 2 × 2 mm silver electrodes, 3 mm apart. Positioning of the electrodes at the spinal outflow to the heart was achieved by applying a continuous square-wave stimulus (25 V, 0·5 ms, 4 Hz) and withdrawing the electrode rod whilst rotating it from side to side until a maximum tachycardia was produced. Serum samples were removed from the aortic arch via a cannula inserted retrogradely in the carotid artery. The cannula was flushed with saline then blood was allowed to flow into the collecting tube. A control was withdrawn simultaneously from the femoral vein with the aid of a syringe.

Time course of DBH release

Blood samples were collected 1, 2, 4 and 8 min following 15 s of stimulation (0·5 ms, 30 V, 4 Hz). These parameters of stimulation had been previously shown to produce the maximum rise in heart rate.

Frequency responses

Blood samples were collected 1 min after a 15 s period of stimulation (0·5 ms, 30 V) at frequencies of 0·5, 1, 2 and 4 Hz.

RESULTS

Mesentery: time course of DBH release

The results indicate that the DBH concentration in the emergent portal vein blood from the mesentery increased significantly ($P < 0·05$) following electrical stimulation of the sympathetic outflow from the T10-L1 level, reaching a maximum of 26·1 nmol ml^{-1}

Table 1. *Post-stimulation changes in the DBH concentration in rat serum taken from various sources following electrical stimulations of selective areas of the spinal cord.* 300 μl samples were collected at various times and the serum assayed for DBH by a spectrophotometric method.

Stimulation region	Stimulation parameters	Serum source	DBH concentration (nmol ml^{-1} h^{-1}) Sampling time (min post stimulation) Cont.	1	2	3	4	6	8	10	16
T10–L1	20 V 0·3 ms 12 Hz 5 s	Splenic vein	19·7 (0·9	19·6 (1·0)		25·2* (1·2)		23·2 (1·3)		19·7 (1·1)	
		Carotid artery	20·1 (1·9)	19·4 (2·1)		18·7 (2·5)		19·2 (1·8)		19·1 (1·7)	
T10–L1	20 V 0·3 ms 12 Hz 5 s	Hepatic portal vein	22·9 (2·1)	24·3 (2·1)	26·1* (1·4)		25·3* (2·3)		23·0 (2·5)		20·9 (1·3)
		Carotid artery	22·4 (2·1)	21·8 (2·4)	16·6 (2·7)		17·8 (2·9)		19·5 (1·9)		20·0 (2·1)
C7–T1	30 V 0·5 ms 4 Hz 15 s	Aortic Arch	9·2 (1·3)	14·8* (1·1)	11·3 (1·3)		8·8 (1·4)		9·8 (1·6)		
		Femoral vein	10·1 (1·1)	8·9 (0·9)	9·9 (1·4)		9·1 (0·7)		9·5 (1·3)		

*$P \leqslant 0{\cdot}05$, n = 6.

h^{-1} at 2 min and returning to prestimulation concentrations (23·0 nmol ml^{-1} h^{-1}) by 8 min (Table 1).

The concentration of DBH in the arterial samples (Table 1) decreased after stimulation reaching a minimum approximately 4 min before returning to prestimulation concentrations.

Frequency responses

The amount of DBH released from the sympathetic nerve endings in the mesentery alone, was determined by subtracting the concentration of DBH in the control arterial sample from that in the blood from the hepatic portal vein. The concentration of DBH released was found to increase with increases in the frequency of stimulation (Fig. 1A). Changes were in the range of 2·01 nmol ml^{-1} h^{-1} following stimulation at 1 Hz to 14·35 nmol ml^{-1} h^{-1} at 25 Hz (Table 2).

Spleen: time course of DBH release

The concentration of DBH in the venous perfusate of the spleen increased following stimulation of the spinal outflow at the T10-L1 level reaching a maximum of 25·2 nmol ml^{-1} h^{-1}, 3 min post-stimulation

Table 2. *The concentration of DBH released into the serum by various organs following electrical stimulation of selective areas of the spinal cord.* DBH activity was measured in 300 μl serum samples collected directly from an organ and from the general circulation. The difference between these two values represents the enzyme released by the organ.

Stimulation region	Stimulation parameters	Sampling time (min post stim)	Serum sources	Change in serum DBH concn: A-B (nmol ml^{-1} h^{-1}) Stimulation frequency Hz 0·5	1	2	3	4	6	12	25
			Spleen								
T10–L1	20 V 0·3 ms 5 s	3	(A) Splenic vein (B) Carotid artery		8·1 (1·3)		13·1 (1·5)		18·4 (1·4)	27·7 (3·1)	25·3 (2·3)
			Mesentery								
T10–L1	20 V 0·3 ms 5 s	2	(A) Hepatic Portal vein (B) Carotid artery		2·0 (0·3)		3·6 (0·5)		5·1 (0·5)	10·8 (1·4)	14·3 (0·9)
			Heart								
C7–T1	30 V 0·5 ms 15 s	1	(A) Aortic arch (B) Femoral vein	1·5 (0·6)	2·4 (0·5)	7·6 (1·2)		9·1 (1·7)			

n = 8.

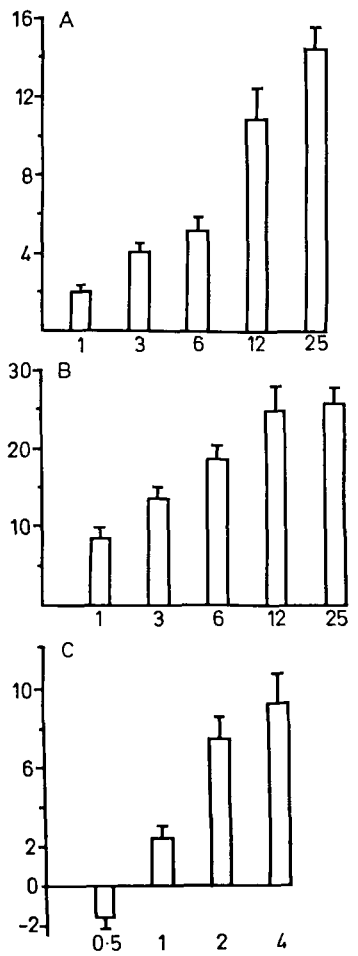

FIG. 1. The effect of stimulation frequency (Hz) (abscissa) on the total amount of DBH released from A—Mesentery (portal venous content—common carotid content). B—Spleen (splenic vein content—common carotid content). The lower sympathetic outflow (T10-L1) of pithed rats was stimulated (20 V, 0·3 ms) for 5 s. n = 8. C—Heart (aortic content—femoral vein content). The cardiac sympathetic outflow (C7-T1) of pithed rats was stimulated (30 V, 0·5 ms) for 15 s. n = 8. DBH concentrations (ordinate) are expressed in terms of the amount of octopamine formed, nmol $ml^{-1} h^{-1}$.

and resuming prestimulation concentrations (19·7 nmol $ml^{-1} h^{-1}$) 10 min after stimulation (Table 1).

Frequency responses

The amount of DBH released from the spleen alone was determined as previously described. The concentration of DBH released was related to the frequency of stimulation (Fig. 1B). 8·47 nmol $ml^{-1} h^{-1}$ followed stimulation at 1 Hz increasing to 25·52 nmol $ml^{-1} h^{-1}$ at 25 Hz (Table 2).

Heart: time course of DBH release

The concentration of DBH in the arterial blood removed from the aortic arch reached a maximum of 14·8 nmol $ml^{-1} h^{-1}$ 1 min post-stimulation and returned to prestimulation concentrations (9·8 nmol $ml^{-1} h^{-1}$) at 8 min (Table 1).

Frequency responses

The amount of DBH released from the heart alone was determined by subtracting the DBH concentration in the venous sample from that of the arterial sample. With the exception of the lowest frequency used (0·5 Hz) the concentration of DBH released by the heart was found to be related to the frequency of stimulation (Fig. 1C). 0·5 Hz caused an overall decrease in DBH activity of 1·33 nmol $ml^{-1} h^{-1}$ however, higher frequencies resulted in an increase, 7·75 nmol $ml^{-1} h^{-1}$ following stimulation at 4 Hz (Table 2).

DISCUSSION

Electrical stimulation of specific segments of the spinal cord induced an increase in the concentration of DBH effluxing from organs known to receive sympathetic innervation from those parts of the cord. Furthermore, the increase in the local serum concentration of DBH was directly related to the frequency of stimulation used. These findings support the hypothesis (Weinshilboum, Nguyen & others, 1971 a) that DBH is released with noradrenaline at sympathetic synapses.

The observed delay between the application of the stimulus and the detection of changes in the concentration of DBH is likely to be related to the rate of diffusion into, and subsequent removal from, the local vascular bed. The enzyme is a protein of high molecular weight so its diffusion away from the synapse is likely to be slow. Thus the maximum change in DBH concentrations from the heart occurred 1 min after stimulation of the appropriate segments of the cord, whereas that in the blood from the spleen or mesentery occurred 2–3 min after stimulation. Venous blood is not the sole vehicle for the removal of DBH from the organ of release. Changes in the concentration of DBH in the lymph of dogs (Ngai, Dairman & others, 1974) and cats (Ross, Eriksson & Hellstrom, 1974) following sympathetic stimulation have been observed. It is thus impossible to draw detailed conclusions with regard to the total release of DBH from the synapse during stimulation from the results of these experiments.

The reduction in the concentration of DBH in aortic blood following low frequency (0·5 Hz)

stimulation of the cardio-sympathetic outflow from the cord was unexpected and not mirrored in the results of experiments on the spleen and mesentery. The explanation of this finding may be related to the fact that the sampling point was relatively remote from the site of release. Blood from the cardiac vessels passed to the lungs and back to the heart before reaching the cannula in the aortic arch.

The greater concentrations of DBH released from the spleen following stimulation compared with that from the heart and mesentery are not necessarily indicative of a greater absolute release of DBH into the blood stream from this organ. Measurement of DBH concentration released, in absolute terms, would require a knowledge of the rate of blood flow through the organ under investigation. This parameter was not measured in our experiments.

There is no information on the metabolism and dispersal of DBH released from the sympathetic nerve system that may be used to explain the reduction in the concentration of the enzyme in the general circulation that was observed following stimulation of the spinal cord. Rush & Geffen (1972) showed that the enzyme is removed by internal metabolic degradation but this does not explain the increased rate of loss following stimulation. It would appear that such a process of degradation or active uptake is enhanced or activated by acute electrical stimulation of the spinal cord.

In conclusion, since DBH is released into the organ perfusates of sympathetically innervated tissues following stimulation, and that the concentration is related to the frequency of such stimulation, the sampling techniques described in this paper, if applied to intact animals, might provide a method for measuring the effects of various drugs and procedures on the activity of the sympathetic nervous system.

Acknowledgment

We thank Dr R. J. Stephens for his valuable advice on the formulation of this manuscript.

REFERENCES

Axelrod, J. (1972). *Pharmac. Rev.*, **24**, 233–243.
De Quattro, V. & Miura, Y. (1973). *Am. J. Med.*, **55**, 362–378.
Duch, D. S., Viveros, O. H. & Kirshner, N. (1968). *Biochem. Pharmac.*, **17**, 255–264.
Geffen, L. B. & Livett, B. G. (1971). *Physiol. Rev.*, **51**, 98–157.
Gillespie, J. S. & Muir, T. C. (1967). *Br. J. Pharmac.*, **30**, 78–87.
Kato, T., Kuzuya, H. & Nagatsu, T. (1974). *Biochem. Med.*, **10**, 320–328.
Nagatsu, T. & Udenfriend, S. (1972). *Clin. Chem.*, **18**, 980–983.
Ngai, S. H., Dairman, W., Marchelle, M. & Spector, S. (1974). *Life Sci.*, **14**, 2431–2439.
Ross, S. B., Eriksson, H. E. & Hellstrom, W. (1974). *Acta physiol. scand.*, **92**, 578–580.
Rush, R. A. & Geffen, L. B. (1972). *Circulation Res.*, **31**, 444–452.
Weinshilboum, R. M., Nguyen, B. T., Johnson, D. G., Kopin, I. J. & Axelrod, J. (1971a). *Science*, **174**, 1349–1351.
Weinshilboum, R. M., Kvetnansky, R., Axelrod, J. & Kopin, I. J. (1971b). *Nature*, **230**, 287–288.
Wooten, G. F. & Cardon, P. V. (1973). *Arch. Neurol.*, **28**, 103–106.
Wooten, G. F. & Ciaranello, R. D. (1974). *Pharmacology*, **12**, 272–282.