J. Physiol. (1978), **278**, *pp.* 287–296
Printed in Great Britain

CHANGES IN PLASMA DOPAMINE β-HYDROXYLASE ACTIVITY INDUCED BY STIMULATION OF THE COMPLETE SYMPATHETIC OUTFLOW IN THE PITHED RAT

BY A. G. GARCÍA, F. PELAYO* AND P. SÁNCHEZ-GARCÍA
From the Departamento de Farmacología, Facultad de Medicina Autónoma, Arzobispo Morcillo s/n, Madrid-34, Spain

(*Received 10 October 1977*)

SUMMARY

1. Plasma dopamine β-hydroxylase (DBH) activity, noradrenaline tissue levels and blood pressure were monitored in the pithed rat following electrical stimulation of the complete sympathetic outflow of the spinal cord. Stimulation at 10 or 30 Hz evoked marked increases in mean blood pressure which averaged 121 mmHg and were abolished by phenoxybenzamine.

2. Stimulation for 1 hr at 10 or 30 Hz did not change the noradrenaline content of the spleen nor the catecholamine content of the adrenals, but the heart noradrenaline content was doubled.

3. Plasma DBH activity was increased by 27 % after 1 hr stimulation at 30 Hz, but remained unchanged after stimulation at 10 Hz, or at 30 Hz in phenoxybenzamine-treated rats.

4. We conclude that the pressor responses evoked by sympathetic nerve stimulation are due to the release of noradrenaline probably from adrenergic nerve terminals supplying the entire vasculature, and that acute alterations of circulating DBH activity are not dependent on the rate of catecholamine release evoked by direct electrical stimulation of sympathetic nerves in the whole pithed rat. The rat seems not to be a good model to study circulating noradrenaline and DBH levels as an index of exocytotic noradrenaline release from adrenergic neurones, and therefore of sympathetic activity.

INTRODUCTION

The precise role of the sympathetic nervous system in the genesis of hypertension is controversial (De Champlain & van Ameringen, 1975). The reuptake and metabolism of released noradrenaline complicates the use of plasma noradrenaline levels as an index of adrenergic neuronal activity. In the last few years the possibility of accurately estimating plasma dopamine beta-hydroxylase (DBH) activity (Weinshilboum & Axelrod, 1971*a*) led to the hypothesis that this enzyme could be a good index of sympathetic activity, bearing in mind that such a protein will not be taken up by the adrenergic nerve endings once it was released by exocytosis (Weinshilboum, Thoa, Johnson, Kopin & Axelrod, 1971*b*). However, results are controversial as far as the correlation between sympathetic and plasma DBH activity is concerned

* Present address: L.E.R.S., 58 Rue de la Glacière 6ème étage, Paris, 13, France.

(Schanberg & Kirshner, 1976). In part this could be due to the fact that in most experiments carried out *in vivo* plasma enzymic activity was monitored after reflex stimulation of sympathetic nerves by exposing the animals to different stressing situations. This means of increasing the frequency of discharge of sympathetic nerves is difficult both to control and to quantify.

The aim of the present experiments was to study the possible correlation between hypertensive responses, plasma DBH activity and tissue catecholamine content following sympathetic stimulation of the complete sympathetic outflow of the spinal cord in the pithed rat. This preparation allows the electrical stimulation of the sympathetic nervous system in suitable controlled conditions of voltage, frequency, duration and time of stimulation. In addition, the blood pressure responses can be continuously monitored. A preliminary report of these findings has been published (Pelayo, García & Sánchez-García, 1975).

METHODS

Sprague-Dawley rats of both sexes weighing 250–300 g were anaesthetized with ether and artificially respired through a tracheal cannula. Rats were pithed according to Gillespie, Maclaren & Pollock (1970) by introducing an electrode through the orbit and the foramen magnum in order to stimulate the thoraco-lumbar segment of the spinal cord. A steel rod inserted behind the skull and pushed down between the vertebral column and skin acted as an indifferent electrode. Blood pressure was recorded from one carotid artery by means of a Statham P23AC transducer connected to a Beckman Polygraph. A jugular vein was cannulated for drug administration. Clotting was prevented by injecting heparin, 1000 i.u. I.V. All animals were treated with atropine (1 mg/kg) and D-tubocurarine (1 mg/kg).

The sympathetic outflow of the spinal cord was stimulated electrically with supramaximal voltage by means of a Grass stimulator (Mod. SD5) using 30 sec trains of 5 msec pulses at frequencies of 10 and 30 Hz. Stimulation periods were repeated every minute throughout the experiment.

DBH assay

Blood samples (0·5 ml.) were collected from the carotid artery before and after stimulation in heparinized chilled tubes. The samples were centrifuged at 10000 g for 10 min at 4 °C and the plasma was stored at −20 °C until assayed. Plasma was diluted 10 times at the time of assay with distilled water and aliquots (0·2 ml.) were assayed for DBH activity according to the method of Goldstein, Freedman & Bonnay (1971) with some modifications (García & Kirpekar, 1975). The optimal pH for the first step of the reaction was 5; this step was continued for 1 hr in presence of 10 μmole *N*-ethylmaleimide and 10 n-mole $CuSO_4$ in order to overcome the effect of endogenous inhibitors. The adequate inactivation of enzyme inhibitors was further tested by adding a known amount of a partially purified bovine adrenal DBH to a duplicate of each sample. Recoveries averaged 90% and all data were routinely corrected for recovery. DBH activity is expressed as n-mole of octopamine formed per hr per ml. of plasma (n-mole/hr.ml.). Since basal values of plasma DBH vary widely in different animals, but are constant with time in the same animal, all the experiments were longitudinally designed so that DBH activity after stimulation could be compared to the basal activity obtained before stimulation in the same animal. In this manner paired comparison of the values could be made.

Tissue noradrenaline assay

At the end of each experiment the heart, spleen and adrenals were homogenized in 5 ml. 0·4 N perchloric acid containing 0·1% sodium metabisulphite and 0·05% ethylenediaminetetraacetic acid (EDTA). Adsorption, elution and assay of noradrenaline were carried out following the method of Shellenberger & Gordon (1971). Recovery of standard amounts of noradrenaline in concomitant assays averaged 80%. All values were corrected for recovery and they are

expressed as μg/g wet tissue weight. In the case of the adrenals the total catecholamine contents (noradrenaline plus adrenaline) were estimated directly without intermediate purification on alumina by the fluorimetric method of Anton & Sayre (1962) and expressed in μg/gland pair.

The statistical significance of the difference between means was determined by Student's *t* test for paired or group data.

Drugs administered

The following drugs were administered through the jugular vein in a volume of 0·25 ml. saline: atropine sulphate (1 mg/kg), D-tubocurarine hydrochloride (1 mg/kg), phenoxybenzamine hydrochloride (5 mg/kg), reserpine (2 mg/kg). In addition, some animals were pretreated intraperitoneally with reserpine (10 mg/kg) 24 hr before the beginning of the experiment.

RESULTS

Time course of the pressor responses evoked by sympathetic nerve stimulation

Stimulation of the spinal sympathetic outflow at 10 or 30 Hz evoked a marked increase in mean blood pressure which averaged 120·8 ± 4·8 mmHg (n = 20). Prolonged stimulation of the nerves (30 sec every min) produced a progressive decline of the hypertensive response which was more pronounced at the higher frequency (Table 1). After 1 hr of stimulation the pressor response significantly ($P < 0·01$) decreased by 34 and 72 % at 10 and 30 Hz, respectively.

TABLE 1. Time course of pressor responses induced by sympathetic stimulation in the pithed rat. E.E., control pressor responses secondary to electrical stimulation at 10 or 30 Hz. Reserpine was injected I.V. in a dose of 2 mg/kg at the beginning of stimulation. The magnitude of the increment of the pressor responses after electrical sympathetic stimulation are expressed in mmHg over the basal blood pressure value. Values are means ± S.E. n, number of experiments

Experimental condition	n	Time of stimulation (min) 0	15	30	45	60
E.E. (10 Hz)	5	119·4 ± 4·9	115 ± 7	93·6 ± 10·4	79 ± 10·2	72·6 ± 10·5*
Reserpine (10 Hz)	5	120 ± 3·1	74·2 ± 8·4*‡	52·2 ± 7·3*‡	37·6 ± 5·1*‡	30·6 ± 5·8*‡
E.E. (30 Hz)	6	122·8 ± 4·8	84·7 ± 6·6*†	50·9 ± 3·9*†	37·1 ± 3·8*†	29·1 ± 3·9*†
Reserpine (30 Hz)	4	107 ± 4·9	59·4 ± 5·9*‡	24·7 ± 2·8*‡	3·3 ± 3·6*‡	0

* $P < 0·01$ when compared to the corresponding controls (0 min).
† $P < 0·001$ when compared to E.E. (10 Hz).
‡ $P < 0·05$ when compared to E.E. of similar frequency without drug.

Administration of reserpine (2 mg/kg I.V.) at the beginning of the experiment significantly increased the rate of decline of the pressor response evoked by sympathetic stimulation so that after 1 hr stimulation the hypertensive response was only 30 % of the initial response at 10 Hz and it was abolished at 30 Hz.

Influence of sympathetic stimulation on tissue levels of noradrenaline and the effects of phenoxybenzamine

Since prolonged intermittent electrical stimulation of the sympathetic nerves induced a progressive decline in the pressor response and this decline was markedly accelerated by reserpine (an agent which interferes with the vesicular synthesis and storage of noradrenaline at the nerve terminals) it was desirable to explore the

possibility of any correlation between tissue noradrenaline levels and the magnitude of the electrically evoked pressor response.

Table 2 shows that stimulation of the nerves at 10 Hz during 1 hr increased the noradrenaline content of the heart by 80% ($P < 0.05$) with no concomitant change in the spleen and adrenals. On the other hand, stimulation at 30 Hz also evoked a significant increase of 50% in the heart noradrenaline, a decrease of adrenal catecholamine and no change in the splenic noradrenaline levels. A plot of tissue noradrenaline levels versus pressor responses before and after stimulation at different frequencies showed no correlation. These results indicate that there was no relationship between the tissue levels of noradrenaline and the observed progressive decline in the pressor response elicited by nerve stimulation.

Since it is known that phenoxybenzamine enhances noradrenaline release evoked by nerve stimulation (Gillespie & Kirpekar, 1965) we studied the effects of acute administration of the drug plus electrical nerve stimulation at 30 Hz on tissue noradrenaline levels. Phenoxybenzamine (5 mg/kg, I.V.) almost immediately abolished the pressor response evoked by stimulation of the sympathetic outflow. However, after the drug administration, stimulation at 30 Hz during 1 hr increased the heart noradrenaline content by 100% but it did not affect the splenic noradrenaline content, a similar result to that obtained in the absence of the drug (Table 2).

TABLE 2. Influence of sympathetic stimulation on noradrenaline (NA) and total catecholamines (CA) tissue levels in the pithed rat and the effects of phenoxybenzamine (PBZ). PBZ was administered I.V. in a dose of 5 mg/kg at the beginning of the experiment. Then the sympathetic outflow was electrically stimulated for 30 sec of every min during 1 hr. n, number of animals. Mean values ± S.E. are shown

Frequency of stimulation	n	Drug treatment	NA (μg/g) Heart	NA (μg/g) Spleen	CA (μg/gland pair) Adrenals
—	5	—	0·34 ± 0·05	0·68 ± 0·11	21·24 ± 4·05
10 Hz	5	—	0·61 ± 0·09*	0·74 ± 0·12	22·29 ± 1·73
30 H	3	—	0·52 ± 0·04*	0·61 ± 0·10	13·91 ± 5·46
30 H	5	PBZ	0·66 ± 0·12*	0·64 ± 0·09	12·63 ± 2·22

* $P < 0.05$ when compared to the corresponding non-stimulated control.

Effects of reserpine on tissue levels of noradrenaline after nerve stimulation

In another set of experiments the effect of acute administration of reserpine on the tissue level of noradrenaline was investigated. Table 3 shows that 1 hr after the administration of reserpine (2 mg/kg, I.V.) alone caused a slight decrease of noradrenaline levels in the heart and spleen when compared to control, non-treated animals. Simultaneous administration of the drug plus 1 hr stimulation at 10 Hz or 30 Hz did not further decrease the heart noradrenaline content; however, the increase in heart noradrenaline induced by nerve stimulation in non-treated animals was suppressed by reserpine. In contrast, splenic noradrenaline levels were markedly reduced after reserpine plus nerve stimulation at 10 or 30 Hz ($P < 0.05$). Finally pretreatment with a larger dose of reserpine (10 mg/kg, I.P.) 24 hr before the experiment reduced the tissue levels of all the organs studied to undetectable levels after 1 hr stimulation at 30 Hz.

Influence of sympathetic stimulation on plasma DBH activity and the effects of phenoxybenzamine

Since the release of noradrenaline evoked by sympathetic nerve stimulation in isolated organs is accompanied by the release of DBH (Weinshilboum *et al.* 1971*b*), and since the pithed rat preparation allows the opportunity of stimulating the entire sympathetic outflow, we felt that it would be interesting to stimulate the sympathetic nerves at different frequencies and estimate the subsequent changes, if any, in circulating DBH activity. The basal plasma DBH activity varied from 2·73 to 9·90 n-mole/hr.ml. (see Methods for explanation of these units) in different animals but the activity was constant with time in the same animal. Therefore the data before and after stimulation in the same animal were compared.

TABLE 3. Influence of reserpine on noradrenaline (NA) and total catecholamines (CA) tissue levels in the pithed rat after sympathetic stimulation. Reserpine was administered I.V. in a dose of 2 mg/kg at the beginning of the experiment or I.P. in a dose of 10 mg/kg, 24 hr before the experiment. *n*, number of experiments. Mean values ± S.E. are shown

Frequency of stimulation	*n*	Drug treatment	NA (μg/g) Heart	NA (μg/g) Spleen	CA (μg/gland pair) Adrenals
—	4	Reserpine I.V.	0·23 ± 0·02	0·52 ± 0·07	22·73 ± 1·79
10 H	4	Reserpine I.V.	0·22 ± 0·07	0·23 ± 0·04*	21·03 ± 1·82
30 Hz	4	Reserpine I.V.	0·29 ± 0·05	0·19 ± 0·03*	20·71 ± 1·21
30 Hz	2	Reserpine I.P.	0	0·01	0

* $P < 0·05$ when compared to reserpine-pretreated rats in the absence of stimulation.

TABLE 4. Influence of sympathetic stimulation on circulating dopamine beta-hydroxylase (DBH) activity in the pithed rat and the effects of phenoxybenzamine (PBZ). Experimental design as in Table 2. C, circulating DBH activity before stimulation; S, circulating DBH activity after stimulation. *n*, number of animals. Mean values ± S.E. are shown

Frequency of stimulation (Stimulation time)	*n*	Drug treatment	DBH n-mole/hr.ml. C	DBH n-mole/hr.ml. S	S/C
10 Hz (1 hr)	3	—	2·73 ± 0·57	2·40 ± 0·85	0·87
30 Hz (1 hr)	6	—	4·32 ± 0·81	5·50 ± 1·02*	1·27
30 Hz (½ hr)	3	PBZ	7·27 ± 2·68	5·28 ± 0·54	0·72
30 Hz (1 hr)	5	PBZ	9·90 ± 1·44	9·76 ± 1·64	0·98

* $P < 0·01$ when compared to C.

Table 4 summarizes the results of two groups of experiments in which plasma DBH activity was estimated before and after 1 hr of electrical stimulation of the sympathetic outflow. Stimulation at 10 Hz did not change the circulating DBH activity; if anything, there was a slight decrease. However, stimulation at 30 Hz increased plasma DBH activity by about 25% ($P < 0·01$). It is worth noting that plasma DBH activity did not appreciably change in the same animal after the surgical manipulation involved in setting up the preparation.

On the basis of these results it was interesting to see whether phenoxybenzamine, a drug which markedly enhances the release of noradrenaline and DBH by nerve

stimulation in isolated organs (Johnson, Thoa, Weinshilboum, Axelrod & Kopin, 1971; De Potter, Chubb, Put & Schaepdryver, 1971), could also change the circulating levels of the enzyme in the entire animal. Table 4 shows that stimulation at 30 Hz for 30 min after I.V. injection of phenoxybenzamine (5 mg/kg) in fact decreased the pre-stimulation levels of DBH by 30%. To our surprise, stimulation for a period of 1 hr after phenoxybenzamine treatment did not modify the circulating enzyme activity.

Effects of reserpine on plasma DBH activity after sympathetic nerve stimulation

The administration of reserpine (2 mg/kg, I.V.) at the beginning of the experiment followed by 1 hr stimulation period at 30 Hz caused about 60% increase of plasma DBH activity ($P = 0{\cdot}05$; Table 5). Similarly, reserpine pretreatment (10 mg/kg, 24 hr before the experiment) followed by 1 hr stimulation at 30 Hz increased again the circulating DBH activity by about 40%. It is interesting to note that in these experiments the rise in plasma DBH activity evoked by sympathetic nerve stimulation took place 24 hr after reserpine, in spite of the fact that sympathetically innervated organs and the adrenals were depleted almost by 100% of their endogenous

TABLE 5. Effect of reserpine on circulating dopamine β-hydroxylase (DBH) activity after sympathetic stimulation in the pithed rat. Reserpine was administered I.V. (*a*) in a dose of 2 mg/kg, at the beginning of the experiment; (*b*) I.P. in a dose of 10 mg/kg 24 hr before the experiment or (*c*) intraperitoneally every 2 days, 10 mg/kg, starting 5 days before the experiment. C, circulating DBH activity before stimulation; S, circulating DBH activity after stimulation. *n*, number of experiments. Mean values ± S.E. are shown

Frequency of stimulation (stimulation time)	*n*	Drug treatment	DBH n-mole/hr.ml. C	DBH n-mole/hr.ml. S	S/C
30 Hz (1 hr)	4	Reserpine (*a*)	2·49 ± 0·18	3·95 ± 0·69	1·58
30 Hz (1 hr)	2	Reserpine (*b*)	4·28	6·07	1·41
30 Hz (1 hr)	1	Reserpine (*c*)	3·30	8·84	2·68

catecholamine contents. These data agree well with those obtained previously in our laboratory in the isolated perfused reserpine-treated cat adrenal gland (Dixon, García & Kirpekar, 1975). In this isolated organ containing no catecholamines, there was a marked release of DBH in response to perfusion with acetylcholine.

In one experiment, reserpine (10 mg/kg, I.P.) was given to the animal every 2 days and at the fifth day the sympathetic outflow was stimulated at 30 Hz for 1 h. After this stimulation pattern an increase of circulating DBH up to 168% over the pre-stimulation level was observed.

DISCUSSION

Electrical stimulation of the sympathetic outflow of the pithed rat spinal cord produces a marked rise in arterial blood pressure which is due to the release of noradrenaline from adrenergic nerve terminals (Gillespie & Muir, 1967). Since pressor responses probably involve the entire vasculature and its sympathetic nerve supply and since in the rat circulating DBH seems to be derived mainly from sympathetic nerve terminals (Weinshilboum & Axelrod, 1971*b*), this preparation

is particularly suited to study the release of both noradrenaline and/or DBH from adrenergic nerve terminals into the circulation in the whole animal.

Stimulation of the sympathetic outflow evokes large pressor responses which are due to the release of catecholamines from sympathetic nerves and, to a lesser extent, from adrenal medulla (Gillespie & Muir, 1967). The fact that the pressor responses were abolished by phenoxybenzamine, a blocker of α adrenoceptors, and that the heart and spleen noradrenaline levels after stimulation were markedly decreased in the presence of reserpine, an agent which interferes with the synthesis of noradrenaline in the vesicles (Kirshner, 1962), indicate that stimulation of the spinal sympathetic outflow caused the release of transmitter from sympathetically innervated organs into the circulation. However, the release of noradrenaline was not unequivocally accompanied by a rise in plasma DBH activity after stimulation at 10 or 30 Hz in normal or phenoxybenzamine-treated animals.

Several studies have attempted to correlate acute alterations of sympathetic activity, evoked reflexly by physiologic or pharmacologic stimuli, with changes in plasma DBH concentrations. For example, forced immobilization of rats induced a significant increase in the plasma levels of the enzyme (Weinshilboum, Kvetnansky, Axelrod & Kopin, 1971*a*). However, drugs which enhance or decrease sympathetic nerve activity increased or decreased rat plasma catecholamine levels, whereas DBH activity remained the same (Reid & Kopin, 1974, 1975).

Under our experimental conditions the entire sympathetic outflow was directly stimulated at different frequencies and only at 30 Hz a slight increase in plasma DBH activity was found. In the presence of phenoxybenzamine, stimulation at 30 Hz did not modify plasma DBH activity in spite of the fact that this drug greatly enhances noradrenaline release evoked by nerve stimulation at these frequencies in isolated organs (Gillespie & Kirpekar, 1965; García, Kirpekar & Sánchez-García, 1976) as well as in the whole animal (Reid & Kopin, 1974). These results agree with those of Reid & Kopin (1974, 1975) and indicate that in the rat, acute alteration of plasma DBH activity is not dependent on the rate of catecholamine release evoked by direct electrical stimulation of sympathetic nerves. We have also recently shown that complete depletion of rat adrenal catecholamines evoked by a strong acute neurogenic stimulation (exposure of the animals to a CO_2-enriched gas mixture) was not accompanied by significant changes in tissue DBH activity (Dixon, García & Kirpekar, 1976). A similar dissociation between release of catecholamines and DBH was also observed in the cat adrenal gland (Dixon *et al.* 1975).

Evaluation of DBH activity in plasma has been used in many laboratories as an index of acute changes of sympathetic nerve activity in animals and man, but our experiments, as well as those of Reid & Kopin (1974, 1975) cast some doubts to this concept at least when the rat is used as experimental model. However, these observations may be reconciled, without ruling out the concept of exocytosis, if the secretion of proteins from vesicles storing catecholamines is species dependent. In fact, we have recently shown that neurogenic stimulation of the sympatho-adrenal system by exposing the animals to a CO_2-enriched gas mixture increased the circulating levels of noradrenaline in the rat and the guinea-pig by 600 and 950%, respectively; on the contrary, while plasma DBH activity of the guinea-pig rose by 1000% the rat circulating enzyme levels were unchanged (García, Arnaiz & Horga,

1977). These results indicate that the guinea-pig, an animal with a greater proportion of releasable DBH (García, Horga & Kirpekar, 1976), is a better model to study circulating noradrenaline levels and DBH activity as an index of exocytotic noradrenaline release from adrenergic neurones and therefore of sympathetic activity.

The failure to demonstrate any great stimulation-dependent changes in plasma DBH activity in the rat could conceivably be because the plasma contains sufficiently high basal levels of DBH to mark a rise due to release from the nerve endings. However, there was no correlation between the magnitude of the release produced by stimulation at 30 Hz and the resting levels in individual animals. In addition, the basal levels of DBH are similar in the guinea-pig and the rat, yet the DBH activity of guinea-pig plasma rose by 10 times after sympathetic neurogenic stimulation (García *et al.* 1977).

Considering that after chronic reserpine treatment the adrenals as well as sympathetically innervated organs were completely depleted of their catecholamine stores, one could expect that the presynaptic regulatory mechanisms for noradrenaline (and hence DBH) release are abolished, and therefore that the release of DBH should be enhanced as well as the enzyme circulating levels, after sympathetic nerve stimulation. However, after 24 hr of reserpine treatment the rise of plasma DBH activity after nerve stimulation was about 40 %, a figure only slightly higher than that obtained in normal animals with their full complement of tissue catecholamines. Even though we only made one experiment with chronic treatment with reserpine for 5 days, it is worth noting that stimulation of the sympathetic outflow resulted in a 168 % increase of plasma DBH activity, which indicates that the rise in tissue enzyme activity may contribute more than the presynaptic receptor autoregulatory mechanism to this drastic increase in circulating DBH activity.

We would finally like to comment on the progressive fall with time of the pressor responses evoked by nerve stimulation. The fall could be due to several factors. First, it may be due to progressive exhaustion of the tissue releasable noradrenaline stores. This is unlikely since after 1 hr stimulation at 10 or 30 Hz, even in the presence of phenoxybenzamine, the splenic and adrenal gland catecholamine contents did not change, and in the heart it was significantly raised (probably due to the higher turnover rate that imposed neurogenic stimulation produced, the synthesis rate being higher than release). Secondly, it could be due to the release of acetylcholine, which by acting on presynaptic muscarinic receptors will inhibit the release of noradrenaline (Muscholl, 1973). This is also unlikely, since all animals were pretreated with atropine. Thirdly, it could be the expression of a progressive desensitization of α-adrenoceptors located at the vascular smooth muscle itself. That this is not the case is shown considering that the administration of exogenous noradrenaline evokes a pressor response which is similar during the course of the experiment (Pelayo, 1976). And finally, the decrease of the pressor responses could be mainly due to a failure of transmitter mobilization after prolonged nerve stimulation. In keeping with this, García *et al.* (1976) have shown that prolonged nerve stimulation first activated and then rapidly inactivated the release of noradrenaline from the perfused cat spleen probably by inactivation of the calcium permeability mechanism present in the neuronal membrane.

This work was supported by grants from the Fondo Nacional para el Desarrollo de la Investigación Científica, Presidencia del Gobierno, Seguridad Social, INP, and Fundación Rodríguez Pascual, Madrid, Spain.

REFERENCES

ANTON, A. H. & SAYRE, D. F. (1962). A study of the factors affecting the aluminum oxide-trihydroxy indole procedure for the analysis of catecholamines. *J. Pharmac, exp. Ther.* **138**, 360–375.

DE CHAMPLAIN, J. & VAN AMERINGEN, M. (1975). Adrenergic mechanisms and the etiology of hypertension. In *Central Action of Drugs in Blood Pressure Regulation*, ed. DAVIS, D. S. & REID, J. L., pp. 69–93. London: Pitman Medical.

DE POTTER, W. P., CHUBB, I. W., PUT, A. & DE SCHAEPDRYVER, A. F. (1971). Facilitation of the release of noradrenaline and dopamine-beta-hydroxylase at low stimulation frequencies by alpha-blocking agents. *Arch. Int. Pharmacodyn.* **193**, 191–197.

DIXON, W. R., GARCÍA, A. G. & KIRPEKAR, S. M. (1975). Release of catecholamines and dopamine beta-hydroxylase from the perfused adrenal gland of the cat. *J. Physiol.* **244**, 805–824.

DIXON, W. R., GARCÍA, A. G. & KIRPEKAR, S. M. (1976). Depletion and recovery of catecholamines in the rat adrenal medulla and its relationship with dopamine beta-hydroxylase. *Proc. R. Soc.* B **194**, 403–416.

GARCÍA, A. G., ARNAIZ, J. M. & HORGA, J. F. (1977). Circulating noradrenaline (NA) and dopamine beta-hydroxylase (DBH) and sympathetic activity in the rat guinea-pig. *Sixth Int. Meeting Int. Soc. Neurochem.* Abstract 164, p. 191. Copenhagen.

GARCÍA, A. G., HORGA, J. F. & KIRPEKAR, S. M. (1976). Tissue and plasma dopamine beta-hydroxylase (DBH) activity after reserpine administration to various animal species. *First Meeting Europ. Soc. Neurochem.*, Bath. Abstracts, p. 41.

GARCÍA, A. G. & KIRPEKAR, S. M. (1975). On the mechanism of release of norepinephrine from cat spleen slices by sodium deprivation and calcium pretreatment *J. Pharmac. exp. Ther.* **192**, 343–350.

GARCÍA, A. G., KIRPEKAR, S. M. & SÁNCHEZ-GARCÍA, P. (1976). Release of noradrenaline from the cat spleen by nerve stimulation and potassium. *J. Physiol.* **261**, 301–317.

GILLESPIE, J. S. & KIRPEKAR, S. M. (1965). The inactivation of infused noradrenaline by the cat spleen. *J. Physiol.* **176**, 205–227.

GILLESPIE, J. S., MACLAREN, A. & POLLOCK, D. (1970). A method of stimulating different segments of the autonomic outflow from the spinal column to various organs in the pithed cat and rat. *Br. J. Pharmac.* **40**, 257–267.

GILLESPIE, J. S. & MUIR, T. C. (1967). A method of stimulating the complete sympathetic outflow from the spinal cord to blood vessels in the pithed rat. *Br. J. Pharmac.* **30**, 78–87.

GOLDSTEIN, M., FREEDMAN, L. S. & BONNAY, M. (1971). An assay for dopamine beta-hydroxylase activity in tissues and serum. *Experientia* **27**, 632–633.

JOHNSON, D. G., THOA, N. B., WEINSHILBOUM, R., AXELROD, J. & KOPIN, I. J. (1971). Enhanced release of dopamine-beta-hydroxylase from sympathetic nerves by calcium and phenoxybenzamine and its reversal by prostaglandins. *Proc. natn. Acad. Sci. U.S.A.* **68**, 2227–2230.

KIRSHNER, N. (1962). Uptake of catecholamines by a particulate fraction of the adrenal medulla. *J. biol. Chem.* **237**, 2311–2317.

MUSCHOLL, E. (1973). Muscarinic inhibition of the norepinephrine release from peripheral sympathetic fibres. In *Pharmacology and the Future of Man.* Proc. 5th Int. Congr. Pharmacology, vol. 4, pp. 440–457. Basel: Karger.

PELAYO, F. (1976). The action of tyramine on the hypertensive responses induced by sympathetic stimulation. *Arch. Pharmac. Tox.* **2**, 105–114.

PELAYO, F., GARCÍA, A. G. & SÁNCHEZ-GARCÍA, P. (1975). Circulating dopamine beta-hydroxylase (DBH) in the pithed rat after stimulation of the spinal sympathetic outflow. *Sixth Int. Cong. Pharmac.* 1158 abs, p. 489. Helsinki: Sanomaprint.

REID, J. L. & KOPIN, I. J. (1974). Significance of plasma dopamine beta-hydroxylase activity as an index of sympathetic neuronal function. *Proc. natn. Acad. Sci. U.S.A.* **71**, 4392–4394.

REID, J. L. & KOPIN, I. J. (1975). The effects of ganglionic blockade, reserpine and vinblastine on plasma catecholamines and dopamine-beta-hydroxylase in the rat. *J. Pharmac. exp. Ther.* **193**, 748–756.

SCHANBERG, S. M. & KIRSHNER, N. (1976). Serum dopamine beta-hydroxylase as an indicator of sympathetic activity and primary hypertension. *Biochem. Pharmac.* **25**, 617–621.

SHELLENBERGER, M. K. & GORDON, J. H. (1971). A rapid simplified procedure for simultaneous assay of norepinephrine, dopamine, and 5-hydroxytryptamine from discrete brain areas. *Analyt. Biochem.* **39**, 356–372.

WEINSHILBOUM, R. & AXELROD, J. (1971*a*). Serum dopamine-beta-hydroxylase activity. *Circulation Res.* **28**, 307–315.

WEINSHILBOUM, R. & AXELROD, J. (1971*b*). Serum dopamine-beta-hydroxylase: decrease after chemical sympathectomy. *Science, N.Y.* **173**, 931–934.

WEINSHILBOUM, R. M., KVETNANSKY, R., AXELROD, J. & KOPIN, I. J. (1971*a*). Elevation of serum dopamine-beta-hydroxylase activity with forced immobilization. *Nature, New Biol.* **230**, 287–288.

WEINSHILBOUM, R. M., THOA, N. B., JOHNSON, D. G., KOPIN, I. J. & AXELROD, J. (1971*b*). Proportional release of norepinephrine and dopamine beta-hydroxylase from sympathetic nerves. *Science, N.Y.* **174**, 1349–1351.