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ORIGIN OF GUINEA-PIG PLASMA DOPAMINE β-HYDROXYLASE

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1 Exposure of guinea-pigs to a CO_2-enriched atmosphere (20% CO_2, 25% O_2, 55% N_2) for 1 to 5 h caused a marked, progressive increase of plasma dopamine β-hydroxylase (DBH) activity which reached its peak after 2 h of CO_2 exposure and then gradually decreased. The increase was abolished by mecamylamine administration before exposure to CO_2. Plasma levels of noradrenaline (NA) also increased after CO_2 exposure.

2 Guanethidine administration, before exposure to CO_2, abolished the increase of plasma NA but potentiated the increase of circulating DBH. Phenoxybenzamine injection, before exposure to CO_2, also potentiated the increase of plasma enzyme activity. In both cases, DBH activity was increased to almost 10 times the basal circulating enzyme levels.

3 Injection of 6-hydroxydopamine (6-OHDA) caused a pronounced decrease of DBH activity in the right atrium, thoracic aorta and spleen; the adrenal enzyme activity was unchanged. Exposure to CO_2 of 6-OHDA-treated animals still evoked a dramatic increase of plasma DBH activity comparable to that found in control animals.

4 The increase of plasma DBH activity evoked by exposure to CO_2 of adrenalectomized animals was considerably diminished.

5 These data suggest that in the guinea-pig, the adrenal is the main source of the increase of circulating DBH activity evoked by exposure of the animals to a CO_2-enriched gas mixture.

Introduction

Secretion of catecholamines (CA) from adrenal medulla and sympathetic nerves occurs by exocytosis, a process in which the soluble contents of the storage vesicles are extruded to the exterior of the cell (Smith & Winkler, 1972). The demonstration that the soluble fraction of dopamine β-hydroxylase (DBH), selectively located in synaptic vesicles of adrenergic neurones and in chromaffin granules of the adrenal medulla, is released along with CA and that the enzyme accumulated in the plasma (Weinshilboum & Axelrod, 1971a; Goldstein, Freedman & Bonnay, 1971), led to the suggestion that plasma DBH activity may serve as an index of sympathetic nerve activity.

Using a CO_2-enriched gas mixture as neurogenic stimulus, Arnaiz, García, Horga & Kirpekar (1978) have shown that changes of tissue and plasma CA and DBH is species-dependent. They also showed that the guinea-pig plasma DBH activity increased 10 times after exposure of the animals to CO_2, while the rat plasma DBH levels remained unchanged. Weinshilboum & Axelrod (1971b) concluded that circulating DBH in the rat derived from sympathetic nerve terminals, the adrenal medulla making no contribution.

Since the guinea-pig is a better model than the rat for the study of changes of circulating DBH activity in response to neurogenic stimuli of the sympathoadrenal system (Arnaiz *et al.*, 1978), we decided to take advantage of the dramatic changes of plasma DBH activity seen after subjecting guinea-pigs to an atmosphere enriched in CO_2 in order to elucidate the source of the increase in plasma DBH activity.

Methods

Treatment of animals

Guinea-pigs (400 to 600 g) were used in this study. All animals were housed in a room with controlled temperature (20 to 22°C), and were allowed water and food *ad libitum*. The animals were subjected to the following treatments and manipulations: (a) intraperitoneal (i.p.) injection of mecamylamine (10, 20 and 30 mg/kg), guanethidine (1 mg/kg) or phenoxybenzamine (5 mg/kg); (b) intracardiac (i.c.) injection of 6-hydroxydopamine (6-OHDA), 2 doses of 30 mg/kg at 24 h interval; and (c) bilateral adrenalectomy under urethane (1 g/kg, i.p.) anaesthesia. After adrenal removal or sham-operation, 10 mg/kg of methyl prednisolone was given intraperitoneally. All drugs were dissolved in 0.9% w/v NaCl solution (saline); 6-OHDA

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was dissolved in saline containing 0.01 N HCl and 1 mg/ml ascorbic acid. Control animals were injected with the same volume of vehicle.

CO_2 exposure

A blood sample was taken from guinea-pigs immediately before exposure to a CO_2-enriched atmosphere. Then, 30 min after drug injection (group a), 48 h after the first dose of 6-OHDA (group b) or 30 min after removal of the adrenal glands (group c), the animals were placed for 2 h in a closed chamber which was flushed continuously with a gas mixture consisting of 20% CO_2, 25% O_2, and 55% N_2 (referred to hereafter as CO_2).

Collection of blood samples

In order to assay plasma DBH activity and NA levels, guinea-pigs were lightly anaesthetized with ether before and after CO_2 exposure. One ml of blood was removed by cardiac puncture and placed in chilled heparinized tubes. Blood samples were centrifuged at 12,000 *g* for 10 min. An aliquot of plasma was stored at −50°C and DBH was assayed the following day. Under these conditions, plasma DBH activity was stable for at least one week. Another plasma aliquot was processed on the day of sampling in order to measure NA levels.

Preparation of tissue homogenates

The 6-OHDA-treated animals were killed at the end of CO_2-exposure and the right atrium, thoracic aorta, spleen and adrenal glands quickly removed, weighed and homogenized in 100 volumes (atrium, aorta and spleen) or 5 ml (pair of adrenal glands) of 5 mM Tris buffer, pH 6.8, containing 0.2% Triton × 100. The homogenates were centrifuged at 26,000 *g* for 10 min and the supernatants analyzed for DBH activity on the same day.

Dopamine β-hydroxylase assay

DBH activity of plasma (50 µl aliquots) and homogenate supernatants (200 µl) was assayed according to the procedure described by Molinoff, Weinshilboum & Axelrod (1971). Several concentrations of $CuSO_4$ (2 to 200 µM) were tested for plasma and each tissue homogenate in order to obtain an adequate inactivation of the endogenous inhibitors of the enzyme. Maximum DBH activities were repeatedly found at 32 µM $CuSO_4$ (final concentration in the reaction mixture). The optimal pH of the first step of the reaction was 5; this step was run for 1 h and the second step for 30 min. DBH activity is expressed as nmol h^{-1} ml^{-1} (plasma), nmol h^{-1} g^{-1} (atrium, aorta and spleen) or nmol h per gland pair (adrenals) of octopamine formed from tyramine.

Noradrenaline assay

Plasma NA levels were estimated by the sensitive radioenzymatic assay of Henry, Starman, Johnson & Williams (1975) and expressed as ng/ml plasma.

Statistical differences between means were assessed by Student's *t* test.

Results

Time course of the effect of CO_2 on plasma dopamine β-hydroxylase activity

We have previously shown that exposure of guinea-pigs to an atmosphere enriched in CO_2 caused a drastic increase of their circulating DBH activity (Arnaiz *et al.*, 1978). Therefore, initial experiments were undertaken in order to determine which was the optimal duration of the period of CO_2 exposure of the animals to give a clear and reproducible increase of plasma DBH activity.

Figure 1 shows that exposure of guinea-pigs to 20% CO_2 for 1 to 5 h caused a clear increase of plasma DBH activity which was already significant ($P < 0.01$) after 1 h. DBH activity was maximal at 2 h and then started to decrease, the levels being lower at 3 and 5 h of CO_2-exposure. The decrease observed at 3 and 5 h might be due to the metabolism and/or inactivation of the enzyme. In view of these results, in all subsequent experiments the animals were exposed to CO_2 for 2 h. Exposure of the animals for 2 h to air in the same chamber did not increase the levels of DBH activity (time zero of Figure 1 compared with basal levels obtained in 50 animals).

Effect of mecamylamine on the increase of plasma dopamine β-hydroxylase activity evoked by CO_2 exposure

This group of experiments was designed in order to test whether CO_2 exposure was evoking a rise of circulating DBH activity by a reflex neurogenic stimulus, i.e., by increasing the rate of nerve firing at the sympatho-adrenal system. Since mecamylamine interferes with transmission at the autonomic ganglia and at the splanchnic nerve-adrenal medulla junction, this drug was used in an attempt to demonstrate that CO_2 exposure was an adequate neurogenic stimulus, as we previously showed to be the case in the rat (Dixon, García & Kirpekar, 1976).

Figure 2 shows the effect of injecting guinea-pigs with mecamylamine, 30 min before exposure to CO_2, on circulating DBH activity. It can be seen that

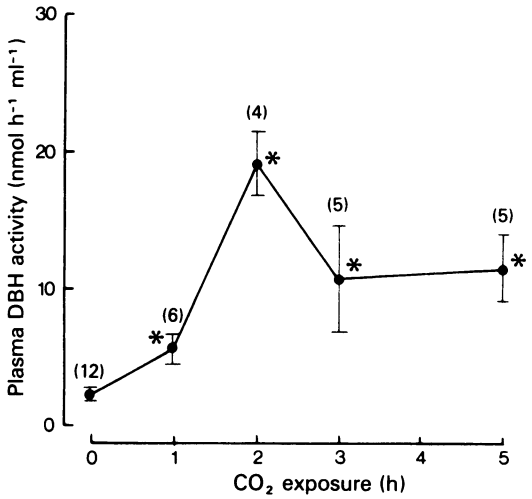

Figure 1 Time course of the effects of CO_2 exposure on guinea-pig plasma dopamine β-hydroxylase (DBH) activity. The animals were exposed to CO_2 for different periods of time. At the time 0, control animals were exposed to air in the same chamber for 2 h. Vertical lines represent s.e. means. Number of animals in parentheses. $*P < 0.001$ compared to air-exposed animals.

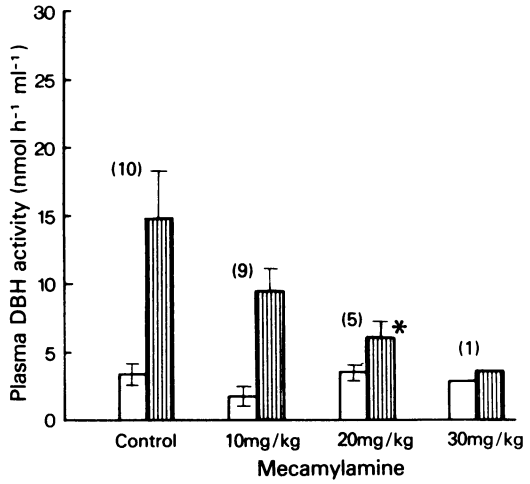

Figure 2 Effects of mecamylamine on the rise of plasma dopamine β-hydroxylase (DBH) activity induced by exposure to CO_2. Mecamylamine was injected i.p. 30 min before CO_2 exposure. Open columns: basal activity; striped columns: activity after CO_2. Vertical lines indicate s.e. means. Number of animals in parentheses. $*P < 0.01$, compared to control.

increasing doses of the drug progressively blocked the increment of plasma DBH activity induced by CO_2 exposure. The blockade was complete at a dose of 30 mg/kg (one experiment). The net increment of DBH activity over basal activity, after CO_2 exposure of animals treated with 10, 20 and 30 mg/kg of mecamylamine, was 71%, 21% and 5% of the increment obtained in control animals (Table 1). These results suggest that CO_2 exposure enhances circulating DBH activity via increased efferent activity of the sympathetic and/or splanchnic nerves.

The effects of guanethidine and phenoxybenzamine on the increase of plasma dopamine β-hydroxylase activity evoked by CO_2 exposure

The experiments with mecamylamine do not provide information as to whether the sympathetic nerve terminals contribute more, equal or less than the adrenals to the overall rise of circulating DBH evoked by CO_2, since this drug interferes with transmission both at the autonomic ganglia and the splanchnico-adrenal junction. Therefore, drugs which specifically act on sympathetic nerves were used.

Guanethidine is known to block the release of NA from adrenergic nerve terminals evoked by electrical stimulation. Injection of 1 mg/kg of this drug to guinea-pigs before exposure to CO_2 abolished the increase of plasma NA evoked by CO_2 (Table 2). However, plasma DBH activity increased above levels obtained in control animals exposed to CO_2 (Figure 3). The net increment of circulating DBH after the combined treatment with guanethidine plus CO_2 was 19.83 ± 2.91 nmol h^{-1} ml^{-1} which amounts to 165% of the increment seen in control animals ($P < 0.05$; Table 1). These data would suggest that under these conditions, the adrenergic nerve terminals contribute little to the rise of plasma DBH activity.

On the other hand, phenoxybenzamine is an adrenoceptor blocking agent which enhances the release of NA and DBH from isolated adrenergically innervated organs in response to sympathetic nerve stimulation. Injection of phenoxybenzamine (5 mg/kg) 30 min before exposure to CO_2 enhanced plasma DBH activity over the increment obtained with CO_2 alone (Figure 3). The net increment of plasma DBH activity after phenoxybenzamine was 19.10 ± 2.55 nmol h^{-1} ml^{-1}, but circulating NA rose only slightly over basal levels (Tables 1 and 2).

Effect of CO_2 exposure on plasma dopamine β-hydroxylase activity of animals treated with 6-hydroxydopamine

Since the two main sources of circulating DBH are, probably, the peripheral adrenergic nerve terminals and/or the adrenal medulla we designed a group of experiments in which chemically sympathectomized animals were exposed to CO_2. Figure 4 shows that a

reasonable degree of sympathectomy was achieved in the right atrium, spleen and thoracic aorta after two injections of 6-OHDA of 30 mg/kg each, at 24 h interval. In all tissues assayed, except the adrenal, DBH activity was considerably decreased (less than 20% of control).

If the rise of plasma DBH observed after CO_2 exposure is mainly due to the enzyme released from adrenergic nerve terminals, then in 6-OHDA-treated animals we should expect a rise quantitatively lower than that found in control animals. However, if the adrenal is an important contributory factor to such a rise, then CO_2 exposure should cause an increase of plasma DBH activity similar to that observed in control animals. That this is the case is shown in Figure 5. The net increment of DBH observed in animals treated with vehicle was 16.27 ± 3.16 nmol h^{-1} ml^{-1} and in animals treated with 6-OHDA 11.46 ± 1.63 nmol h^{-1} ml^{-1} (Table 1). This increment was similar to that obtained in control animals (Figure 2), but it was lower than the increment observed in vehicle-treated animals ($P > 0.05$ in both cases).

It is also interesting to note that the basal plasma DBH activity was lower in 6-OHDA-than in vehicle-

Table 1 Net increment of plasma dopamine β-hydroxylase (DBH) activity over basal levels after exposure to CO_2

Treatment	n	*Net increment of DBH activity* (nmol h^{-1} ml^{-1})	% control
Control	10	12.03 ± 2.67	100
Mecamylamine			
−10 mg/kg	9	8.61 ± 1.50	71.57
−20 mg/kg	5	2.49 ± 0.69**	20.7
−30 mg/kg	1	0.6	4.99
Guanethidine			
(1 mg/kg)	9	19.83 ± 2.91*	164.83
Phenoxybenzamine			
(5 mg/kg)	6	19.19 ± 2.55*	159.52
6-OHDA			
(2 × 30 mg/kg)	14	11.46 ± 1.63	95.26
Sham-operated	8	15.25 ± 2.03	126.77
Adrenalectomy	8	4.24 ± 1.37***	35.25

The net increment was calculated by subtracting the value of DBH activity before CO_2 exposure from the value obtained after CO_2 exposure. Data are mean ± s.e.; *n*, number of animals.
*$P < 0.05$; **$P < 0.01$, compared to control. ***$P < 0.001$, compared to sham-operated.

Table 2 Net increment of plasma noradrenaline (NA) over basal levels after exposure to CO_2

Treatment	n	*Net increment of NA* (*ng/ml*)	% *control*
Control	10	6.62 ± 0.46	100
Mecamylamine			
−10 mg/kg	9	0.74 ± 0.08*	11
−20 mg/kg	4	1.58 ± 0.33*	24
−30 mg/kg	1	8.7	131.51
Guanethidine			
(1 mg/kg)	9	0.097 ± 0.01*	1
Phenoxybenzamine			
(5 mg/kg)	6	1.03 ± 0.14*	16
6-OHDA			
(2 × 10 mg/kg)	4	1.85 ± 0.33*	28
Sham-operated	4	6.35 ± 1.14	95.89
Adrenalectomy	8	7.50 ± 0.52	113.24

The net increment was calculated as in Table 1. Data are mean ± s.e.; *n*: number of experiments.
*$P < 0.01$, compared to control.

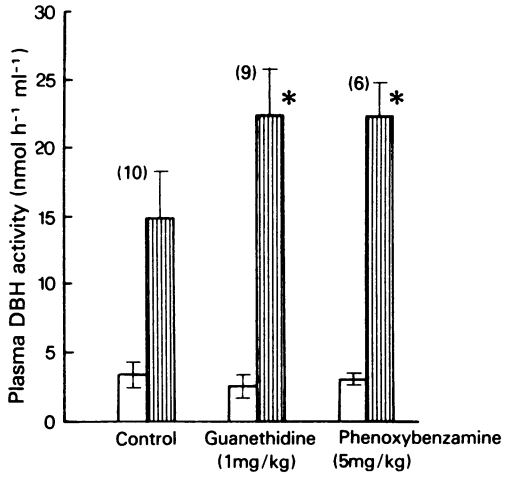

Figure 3 Effect of guanethidine and phenoxybenzamine on the rise of plasma dopamine β-hydroxylase (DBH) activity induced by exposure to CO_2. Guanethidine and phenoxybenzamine were injected, i.p. 30 min before exposure to CO_2. Open columns: basal activity; striped columns: activity after CO_2. Vertical lines represent s.e. means. Number of animals in parentheses. *$P < 0.05$, compared to control.

treated animals ($P > 0.05$), but that it was similar to that found in control animals.

Effect of CO_2 exposure on plasma dopamine β-hydroxylase activity of adrenalectomized animals.

In order to assess the role of the adrenal as a source of the rise of plasma DBH activity evoked by CO_2 exposure, both adrenal glands were removed from a group of guinea-pigs which were then exposed to CO_2.

Basal DBH activity, immediately after the operation and before CO_2 exposure in sham-operated and adrenalectomized animals was considerably higher than basal DBH activity seen in control animals (compare open columns of Figures 2, 3 and 6). This increase is probably due to the stress of the operation and manipulation of the adrenal gland during the rather complicated surgical procedure in this animal.

It is clear that in adrenalectomized animals the net increment of circulating DBH activity was only 35% of controls (26% of the increment obtained in the sham operated group, $P < 0.001$) while in the sham-operated group the increment was 127% of that found in control animals exposed to CO_2 (Figure 6; Table 1).

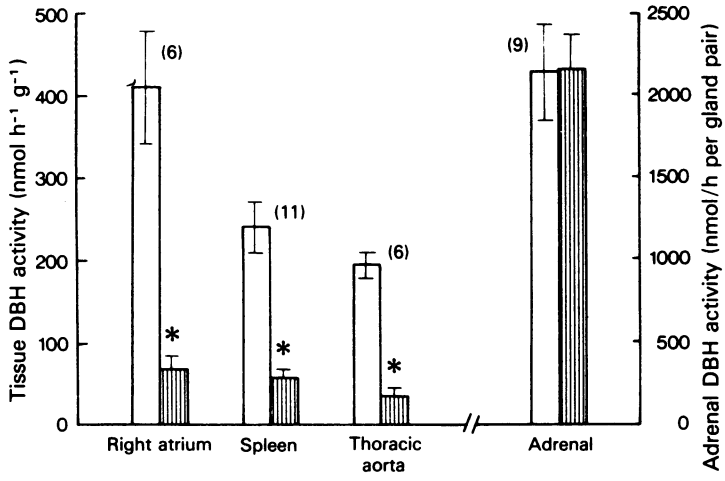

Figure 4 Effect of 6-hydroxydopamine (6-OHDA) on tissue dopamine β-hydroxylase (DBH) activity of guinea-pig right atrium, spleen, thoracic aorta and adrenal. Two intracardiac doses (30 mg/kg each) of 6-OHDA, were injected at 24 h intervals. Control animals were injected with the same volume of vehicle. Open columns: control animals; striped columns: after 6-OHDA. Vertical lines represent s.e. means. Number of animals in parentheses. *$P < 0.01$, compared to vehicle-injected animals.

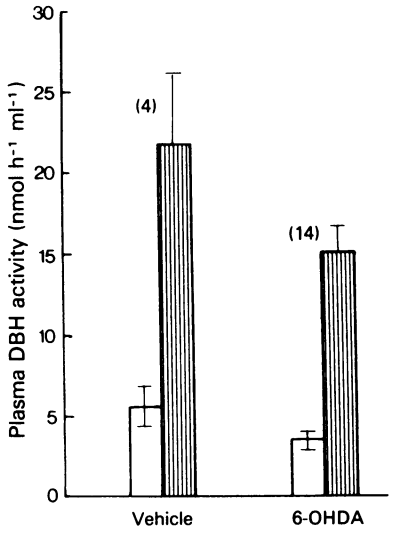

Figure 5 Effect of 6-hydroxydopamine (6-OHDA) on the rise of plasma dopamine β-hydroxylase (DBH) activity induced by exposure to CO_2. Administration of 6-OHDA and vehicle was carried out as indicated in Figure 4. Forty-eight h after the first dose of 6-OHDA, the animals were exposed to CO_2. Open columns: basal activity; striped columns: activity after CO_2. Vertical lines indicate s.e. means. Number of experiments in parentheses.

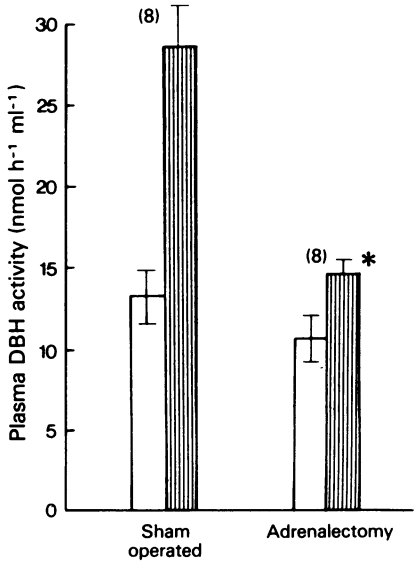

Figure 6 The effects of exposure to CO_2 on plasma dopamine β-hydroxylase (DBH) activity of adrenalectomized and sham-operated guinea-pigs. Thirty min after the operation, the animals were exposed to CO_2. Open columns: basal activity; striped columns: activity after CO_2. Vertical lines indicate s.e. means. Number of animals in parentheses. $*P < 0.001$, compared to sham-operated.

Discussion

The present experiments show that exposure of guinea-pigs to CO_2 causes a significant increase of plasma DBH activity which apparently has its primary source in the adrenal medulla. Recently, Cubeddu, Santiago, Talmaciu & Pinardi (1977) have also indicated that the adrenal gland contributes almost exclusively to the rise in plasma DBH and CA caused by bleeding stress in the dog.

Previous studies in our laboratory have shown that exposure of rats to CO_2 enhances the flow of impulses through the sympatho-adrenal system, since the CA-releasing effect of CO_2 exposure is abolished in splanchnicotomized or mecamylamine treated animals (Dixon *et al.*, 1976; Arnaiz *et al.*, 1978). The present results demonstrate that this is also the case for the guinea-pig, since increasing doses of mecamylamine progressively blocked the increase of plasma DBH activity induced by CO_2 exposure.

Plasma DBH activity increased after CO_2 exposure and reached a peak after 2 h of treatment. From this time onwards the increase of circulating enzyme was smaller. This fact can be due to inactivation of the enzyme after its release, or its degradation once it reaches the circulation. Virtually nothing is known about the metabolism of circulating DBH, but attempts made to estimate the half life of the plasma enzyme have yield values of 3 h in the sheep, 6 h in the mouse, 8 h in human and several days in the rat (Schanberg and Kirshner, 1976). Recently, Grzanna & Coyle (1977) have shown that after supression of the circulating enzyme activity by injecting DBH antibodies to the rat, the half life of DBH, estimated from the rate of appearance of the enzyme into the circulation, was 4.2 days.

After exposure of phenoxybenzamine-treated guinea-pigs to CO_2, an increase of 7 times basal plasma DBH activity was observed. In order to estimate the half life of guinea-pig circulating DBH we are now following the rate of decay of the enzyme activity in plasma after CO_2 exposure. Preliminary results indicate that the half life of plasma DBH in the guinea-pig is about 3 h (unpublished results). This observation correlates well with the present results which indicate that CO_2 exposure causes a maximal increment of circulating DBH at 2 h, probably because after this time the rate of inactivation of the circulat-

ing enzyme is greater than the rate of its release from the tissues.

Source of the increase of circulating dopamine β-hydroxylase after CO_2 exposure

The selective pharmacological manipulation of the adrenergic neurone with drugs which interfere with its activity (guanethidine, phenoxybenzamine) or destroy the adrenergic nerve terminals (6-OHDA) provided evidence which suggests a primary role of the adrenal medulla as a tissue source of the increase of plasma DBH activity seen after acute exposure of guinea-pigs to CO_2. Weinshilboum & Axelrod (1971b) demonstrated that the basal serum DBH activity was reduced 18 to 25% after giving to rats two injections of 6-OHDA 24 h apart. Since adrenal demedullation did not modify serum DBH activity, the authors concluded that the source of at least a portion of the serum DBH activity is the sympathetic nerve terminal.

It is known that the DBH assay method has some sensitivity limitations when measuring a decrease of animal plasma enzyme activity. However, under our experimental conditions and due to the fact that CO_2 exposure causes a dramatic increment in plasma DBH activity (10 times the basal levels) the changes in DBH activity can more reliably be measured.

The results of our experiments show that 48 h after the first 6-OHDA injection, when the tissue DBH activity was diminished by over 80%, the basal plasma DBH activity was not significantly decreased as compared to control, non-treated animals, even though there was some decrease ($P > 0.05$) of the basal levels when compared with vehicle-injected animals. However, in this last group of animals (see Figure 5), the basal enzymatic levels might be raised as a consequence of stress caused to the animal during intracardiac injection of vehicle. An attempt to inject 6-OHDA to adrenalectomized animals was carried out but the guinea-pig is very sensitive to the adrenal removal, even if methyl prednisolone was administered, and all animals died.

We have previously demonstrated that total tissue DBH activities were much higher in the guinea-pig than in the rat (Arnaiz *et al.*, 1978). Similar results have been reported by Kato, Wakui, Nagatsu & Ohnishi (1978). We have also shown that the ratios of total DBH activity to CA in the spleen and adrenal were much higher in the guinea-pig than in the rat. The fraction of total DBH activity that can be solubilized by osmotic shock of purified adrenomedullary chromaffin granules was 28% in the rat and 71% in the guinea-pig. If one makes the assumption that 'soluble' and 'releasable' DBH may be equated, the amount of adrenal 'releasable' DBH into the circulation is 102 u per ml plasma in the guinea-pig, and only 2.8 u/ml plasma in the rat (Arnaiz *et al.*, 1978). With these data in mind, and considering that the basal circulating activity in the guinea-pig and rat is similar (Arnaiz *et al.*, 1978) it is obvious that the guinea-pig, but not the rat, will easily respond to neurogenic stimuli with a sharp increase of the circulating DBH activity.

On the basis of the DBH assay limitations discussed above and taking into account the different tissue activity and subcellular distribution of the enzyme in the guinea-pig and rat, we felt that in order to explore the source of the increased plasma enzymatic activity, the increase of the rate of discharge of CA and DBH from the tissues into the circulation, induced by CO_2 exposure, might be a better approach, than simply measuring the basal levels of the circulating enzyme.

If the adrenal is responsible for the sharp rise of DBH activity seen after CO_2 exposure, then this increase should be diminished, or abolished, after bilateral adrenalectomy. And this was indeed the case in the guinea-pig. In fact the net increment of plasma DBH activity over basal levels, after exposing adrenalectomized animals to CO_2, was diminished by over 70% when compared to the net increment obtained in sham-operated animals (Table 1). However, in adrenalectomized animals there was still present some increase of plasma DBH activity which may originate from sympathetically innervated tissues.

The fact that exposure of 6-OHDA-treated animals to CO_2 resulted in an increase of circulating enzyme, similar to control animals, gives additional support to the idea that the adrenal is almost exclusively the source of the plasma DBH increase seen after neurogenic stimulation of the sympathoadrenal system and may consequently also contribute to the maintenance of the basal levels of the enzyme in the blood.

Finally, the sharp rise of plasma DBH evoked by CO_2 exposure in animals treated with guanethidine and phenoxybenzamine, without a concomitant increase of the levels of plasma NA, also indicates that the enzyme has its origin in the adrenal, since it is well known that these drugs act mainly on the adrenergic nerve terminals, and not in the adrenal medulla (Kirpekar, 1975).

The potentiation of the rise of plasma DBH activity seen after guanethidine may be due to a reflex, augmented discharge of the adrenal, in order to compensate the hypotension produced by sympathetic neurotransmission blockade at the vascular neuro-effector junction; in the case of phenoxybenzamine, its well known blocking effects on post- and presynaptic α-adrenoceptors can contribute to the overall effect (Kirpekar, 1975).

From all these results, we conclude that in the guinea-pig, an animal species which has a high level of 'releasable' tissue DBH (Arnaiz *et al.*, 1978), the adre-

nal medulla greatly contributes to the presence of DBH in the blood. The contribution of the adrenergic nerve terminals to the rise of circulating DBH evoked by exposure of the animals to CO_2 seems to be of minor importance.

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References

ARNAIZ, J.M., GARCÍA A.G., HORGA, J.F. & KIRPEKAR, S.M. (1978). Tissue and plasma catecholamines and dopamine β-hydroxylase activity of various animal species after neurogenic sympathetic stimulation. *J. Physiol.*, **285**, 515–529.

CUBEDDU, X.L., SANTIAGO, E., TALMACIU, R. & PINARDI, G. (1977). Adrenal origin of the increase in plasma dopamine β-hydroxylase and catecholamines induced by hemorrhagic hypotension in dogs. *J. Pharmac. exp. Ther.*, **203**, 587–597.

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 β-hydroxylase. *Proc. R. Soc. Lond. B.*, **194**, 403–416.

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

GRZANNA, R. & COYLE, J.T. (1977). Immunochemical studies on the turnover of rat serum dopamine β-hydroxylase. *Molec. Pharmac.*, **13**, 956–964.

HENRY, D.P., STARMAN, B.J., JOHNSON, D.G. & WILLIAMS, R.H. (1975). A sensitive radioenzymatic assay for norepinephrine in tissues and plasma. *Life Sci., Oxford*, **16**, 375–384.

KATO, T., WAKUI, T., NAGATSU, T. & OHNISHI, T. (1978) An improved dual-wavelength spectrophotometric assay for dopamine-β-hydroxylase. *Biochem. Pharmac.*, **27**, 829–831.

KIRPEKAR, S.M. (1975). Factors influencing transmission at adrenergic synapses. *Prog. Neurobiol.*, **4**, 163–210.

MOLINOFF, P.B., WEINSHILBOUM, R. & AXELROD, J. (1971). A sensitive enzymatic assay for dopamine β-hydroxylase. *J. Pharmac. exp. Ther.*, **178**, 425–431.

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

SMITH, A.D. & WINKLER, H. (1972). Fundamental mechanisms in the release of catecholamines. In *Catecholamines*. ed. Blaschko, H. & Muscholl, E. pp. 538–617. Berlin: Springer Verlag.

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

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

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