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Effects of Chemical Sympathectomy on the Increases in Plasma Catecholamines and Dopamine-β-Hydroxylase Induced by Forced Immobilization and Insulin-Induced Hypoglycemia: Origin and Fate of Plasma Dopamine-β-Hydroxylase[1]

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ABSTRACT

Israel, A. S., Y. R. Barbella and L. X. Cubeddu: *Effects of chemical sympathectomy on the increases in plasma catecholamines and dopamine-β-hydroxylase induced by forced immobilization and insulin-induced hypoglycemia: Origin and fate of plasma dopamine-β-hydroxylase.* J. Pharmacol. Exp. Ther. **221:** 577–583, 1982.

The effect of acute stresses on plasma norepinephrine, epinephrine and dopamine-β-hydroxylase (DBH) were evaluated in control and 6-hydroxydopamine-treated, awake cannulated guinea pigs. Forced immobilization for 1 hr caused a 3- and 5-fold increase in plasma DBH and norepinephrine, respectively. Pretreatment with 6-hydroxydopamine (23 mg/kg b.wt. i.a., 72 and 48 hr before stress) reduced by 70% the increase in plasma DBH and totally prevented the rise in plasma catecholamines evoked by the restraining stress. Injection of insulin (5 U/kg b.wt. i.a.) induced a 60% decrease in blood glucose, a 1-fold increase in plasma DBH and a selective 4-fold increase in plasma epinephrine; these effects were not modified by chemical sympathectomy. Our results indicate that forced immobilization and hypoglycemia produce a preferential activation of the sympathetic postganglionic nerves and of the adrenal medulla, respectively, and that in guinea pigs both stresses increase plasma DBH. The kinetics of disappearance of plasma DBH were studied after subjecting the guinea pigs for 1 hr to forced immobilization. Although 7 of 12 animals showed a biphasic rate of fall of plasma DBH, in each case there was a rapid initial fall possibly due to the "distribution" of the enzyme with a $T_{1/2}$ of 1.65 hr. Similar findings were observed in 6-hydroxydopamine-treated guinea pigs. These results suggest that the distribution of DBH is the most important process in reducing the augmented plasma DBH levels elicited by a short-term stress and that this process is not dependent on the integrity of the sympathetic nerves nor on the adrenal or sympathetic origin of the enzyme. This study supports the view that the ratio, content of releasable DBH present in sympathetic nerves and adrenal glands/total circulating pool of DBH, is the factor that determines whether an increase in plasma DBH would occur in animals exposed to an acute stress.

Measurements of plasma CA and DBH have been employed to evaluate the increases in sympatho-adrenal activity induced by stress (Kvetnansky and Mikulaj, 1970; Weinshilboum *et al.*, 1971; Roffman *et al.*, 1973; Mueller *et al.*, 1969; Reid and Kopin, 1974, 1975; Planz *et al.*, 1975; Cubeddu *et al.*, 1977, 1979; Arnaiz *et al.*, 1978, 1980; Garcia *et al.*, 1978; Kvetnansky *et al.*, 1978; Grzanna and Coyle, 1978). In these studies, the increases in plasma CA occurred at earlier times and were of greater magnitude than those of DBH. In addition, it has been observed that although an elevation in plasma CA is a consistent finding in animals exposed to stress, the increase in plasma DBH is highly dependent on the animal species employed (Cubeddu *et al.*, 1977, 1979; Arnaiz *et al.*, 1978, 1980).

Recent studies revealed that guinea pigs are the best animal model for studying the effects of stress on plasma DBH and for monitoring through changes in the plasma enzyme levels the degree of sympatho-adrenal activation (Arnaiz *et al.*, 1978, 1980; Cubeddu *et al.*, 1979). In this regard, Cubeddu *et al.*, (1979) reported that the higher ratio, content of adrenal soluble DBH/plasma content of DBH found in guinea-pigs, is responsible for the much larger increases in plasma enzyme levels observed in these rodents when exposed to stresses which increase the firing of the splanchnic nerves. However, these authors did not rule

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ABBREVIATIONS: CA, catecholamine; DBH, dopamine-β-hydroxylase; FI, forced immobilization; 6-OHDA, 6-hydroxydopamine; IIH, insulin-induced hypoglycemia; NE, norepinephrine; EPI, epinephrine.

out the possibility that the greater increases in plasma DBH found in guinea pigs could have been due to differences in the kinetics of degradation of plasma DBH. Consequently, in the present study, we followed the rate of disappearance of the concentration and specific activity of plasma DBH in guinea pigs whose basal plasma DBH levels had been increased by FI. The kinetic behavior of plasma DBH in controls and 6-OHDA-treated animals was also investigated.

In addition to the animals species employed, the type of stress applied could also determine the magnitude of the increase in plasma DBH induced by a certain stress (Cubeddu, 1980). To evaluate this possibility, we studied the effects of FI and of IIH on the plasma levels of NE, EPI and DBH in awake, cannulated guinea pigs. These experiments were performed both in normal (controls) and 6-OHDA-treated animals. Information was gained with respect to the use of plasma DBH, NE and EPI as indicators of acute changes in sympathetic or adrenal medullary discharge, as well as to determine the origin of the increases in plasma DBH and CA induced by these acute stresses.

Materials and Methods

Arterial cannulation. Male guinea pigs, weighing 350 to 600 g, were anesthetized with sodium pentobarbital (30 mg/kg b.wt. i.p.); subsequently, the left carotid artery was dissected and cannulated with a Teflon catheter no. 20 which was connected to a silicone tubing of 15 cm and filled with a saline solution containing 10 U of heparin per ml. The tubing was then passed underneath the skin and brought out to the surface through a small incision in the intrascapular region. A stainless-steel extension spring (20 cm) was used to protect the tubing so that the guinea pig could not bite the cannula. Ampicillin (25 mg) was applied locally and the skin suture was covered with bacitracin ointment. The cannula was filled and flushed daily with sterile saline solution containing 20 U/ml of heparin.

Blood samples were obtained after removing the solution filling the dead space of the cannula (about 0.3 ml) along with some blood. In general, samples of 0.3 to 0.4 ml of blood were drawn for chemical determinations. Subsequently, a similar volume of saline was returned to the guinea pig and the dead space was refilled with the heparinized saline solution. All guinea pigs were cannulated 72 hr before being subjected to stress.

FI stress. Cannulated, awake guinea pigs were immobilized on their backs for 1 hr by securing their legs to a metal board. The head motion was limited by one metal loop fixed over the neck area. The stress was always initiated between 8 and 9 A.M. Blood samples for the determination of CAs, DBH and proteins were drawn before, during and after the stress procedure. Four samples were obtained during FI at 10, 20, 30 and 60 min and eight additional samples were drawn after returning the animals to their cages. Cannulated, awake guinea pigs, from whom blood samples were removed as described above but not exposed to FI, served as controls.

Hypoglycemic stress. Cannulated, awake guinea pigs, fasted for 24 hr, were injected with crystalline insulin (5 U/kg b.wt. i.a.) and samples of 0.4 ml of blood each were drawn for the determination of CA, DBH, glucose and proteins, before and at 30, 60, 90 and 120 min after insulin administration.

Chemical sympathectomy. Seventy two hours before stress and immediately after the left carotid artery was cannulated, a group of guinea pigs received an i.a. dose of 23 mg/kg b.wt. of 6-OHDA, which was repeated on the next day. The content of CA and DBH of the heart, spleen and adrenal medulla was determined in control and 6-OHDA-treated guinea pigs 72 hr after cannulation; none of these animals were subjected to FI or hypoglycemic stress nor to serial removals of blood. The organs were removed under pentobarbital administration (30 mg/kg b.wt. i.a.).

Blood samples. Blood samples were collected in ice-cold polypropylene tubes containing heparin (10 U) and 10 μl of a pH 6.5 solution of 0.16 M reduced glutathione and 0.31 M ethyleneglycol bis-(*b*-aminoethyl ether)-N-N^1-tetracetic acid. Subsequently, the samples were centrifuged at 27,000 × *g* for 10 min at 2°C and the plasma was stored at −40°C for CA and DBH determinations.

DBH determinations. DBH activity was determined by the procedure of Molinoff *et al.* (1971) as described in detail by Cubeddu *et al.* (1977). The enzymatic activity was expressed in nanomoles or micromoles of octopamine formed per hour. Appropriate dilutions of the samples with ice-cold distilled water and different copper concentrations were employed to determine the conditions for optimal enzyme activity. In general, the plasmas were diluted 1:12 (v/v) and assayed for 60 or 120 min during the first step (DBH step), employing 5 μM $CuSO_4$. The spleen, heart and adrenal gland supernatants were diluted 1:10, 1:20 and 1:200, respectively, and 10, 15 and 2.5 μM $CuSO_4$ were used, respectively. The tissue samples were incubated for 10 min during the DBH step. Complete standard curves with octopamine (6–200 pmol) were included in each assay. The plasma samples were assayed in triplicate and the variability between replicates was of 5.6 ± 1.4% (n = 36). Twice, blank values were obtained with 12.5 pmol of octopamine. The sensitivity of the assay was also expressed as the amount of [^{3}H] synephrine formed (240 ± 18 dpm) per picomole of octopamine.

To determine the rate of fall of plasma DBH, the plasma samples of each guinea pig were assayed together. In 6-OHDA-treated animals, additional assays were performed to compare the basal (prestress) DBH activity with that present in samples collected after the 5th hr poststress. In these assays, the DBH step was incubated for 2 hr (linearity up to 2.5 hr).

CA determinations. Plasma and tissue CAs (NE and EPI) were determined by a modification of the radioenzymatic procedure of Da Prada and Zurcher (1976) described in detail by Cubeddu *et al.* (1979). The CA levels were expressed as nanograms per milliliter of plasma or micrograms per organ. Standard curves from 0.25 to 10 ng of each CA were routinely employed in the calculations. Double blank values were obtained with approximately 150 pg of each amine.

Other determinations. Plasma glucose was determined by a quantitative enzymatic assay based on the use of hexokinase and glucose-6-phosphate dehydrogenase as described by Barthelmai and Czok (1962). Proteins were determined by the method of Lowry *et al.* (1951) using bovine serum albumin as standard.

Kinetic calculations were performed by the method of feathering described in detail by Gibaldi and Perrier (1975). The area under the curve was calculated employing the trapezoidal technique (Gibaldi and Perrier, 1975). Mean values ± S.E.M. were calculated from individual values. Statistical calculations were performed according to conventional procedures (Snedecor and Cochran, 1969).

Results

Effects of FI and IIH on plasma DBH and CAs in awake-cannulated guinea pigs. Male guinea pigs were cannulated 72 hr before being subjected to either FI or IIH. The basal plasma DBH and CA levels were estimated from blood samples taken just before subjecting the animals to either stress (table 1); in these samples the concentrations of NE were 3 times greater than those of EPI (table 1; fig. 1). Basal plasma DBH activity was expressed both as concentration (nanomoles per hour per milliliter) and as specific activity (picomoles per hour per milligram of protein). The latter corrects for possible changes in plasma volume induced by acute stress.

FI induced marked increases in the plasma levels of DBH and CAs (fig. 1); however, whereas the CAs showed a rapid rise to a sustained elevated level, a progressive increase in plasma DBH concentration and specific activity occurred throughout the stress period. In addition, after termination of the FI period,

TABLE 1

Basal plasma levels of DBH, NE and EPI in control and 6-OHDA-treated guinea pigs

Plasma DBH and CAs were determined in blood samples obtained from awake cannulated guinea pigs either untreated (controls) or pretreated with 6-OHDA, just before being subjected to stress. All samples were withdrawn between 8 and 9 A.M. DBH activity is expressed as concentration (nanomoles per hour per milliliter) and as specific activity (picomoles per hour per milligram of protein[1]). Shown are mean values ± S.E.M. of *n* experiments.

	DBH		NE	EPI
	nmol hr^{-1} ml^{-1}	*pmol hr^{-1} mg protein^{-1}*	*ng ml^{-1}*	*ng ml^{-1}*
Controls	4.33 ± 0.50 (*n* = 20)	101 ± 9 (*n* = 18)	2.35 ± 0.32 (*n* = 16)	0.97 ± 0.21 (*n* = 15)
6-OHDA	5.53 ± 0.48 (*n* = 13)	126 ± 11 (*n* = 13)	1.25 ± 0.22* (*n* = 11)	1.05 ± 0.25 (*n* = 10)

* $P < .05$.

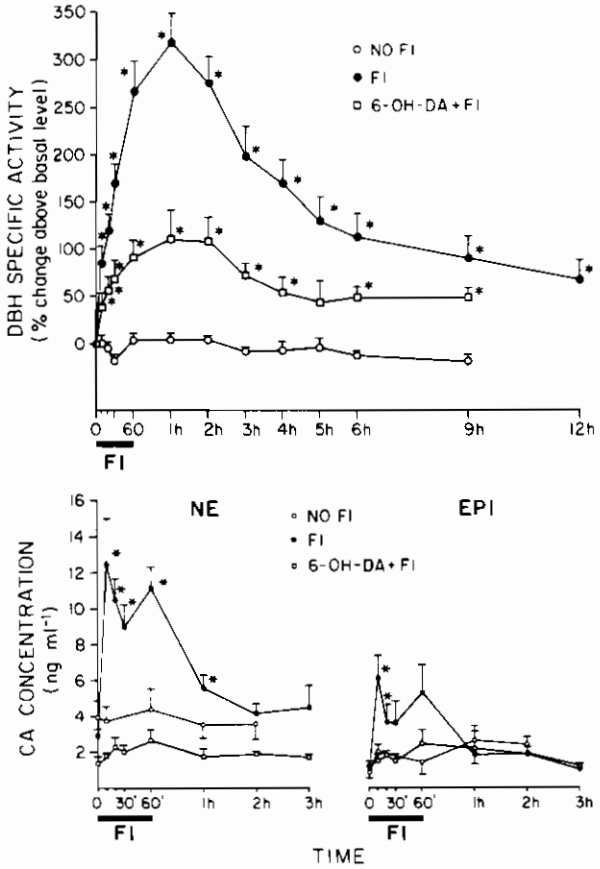

Fig. 1. Effects of FI on plasma DBH and CA in awake cannulated control and 6-OHDA-treated guinea pigs. The graphs show the changes in specific activity of plasma DBH (superior) and plasma concentration of NE (left inferior) and EPI (right inferior) in normal (●) (n = 12) and 8-OHDA-treated animals (□) (n = 7) exposed to FI for 1 hr. Control animals not exposed to FI (n = 5) from which blood samples were removed for previous groups showed no significant changes in plasma DBH (○) and CA (○). Ordinates: specific activity of plasma DBH (picomoles per hour per milligram of protein) as percentage of change above control levels; NE and EPI plasma concentrations in nanograms per milliliter. Abscissa: time in minutes and hours. The bar shown beneath indicates the duration of the FI period. Shown are mean values ± S.E.M. of n experiments. * Significantly different from control at at least $P < .05$.

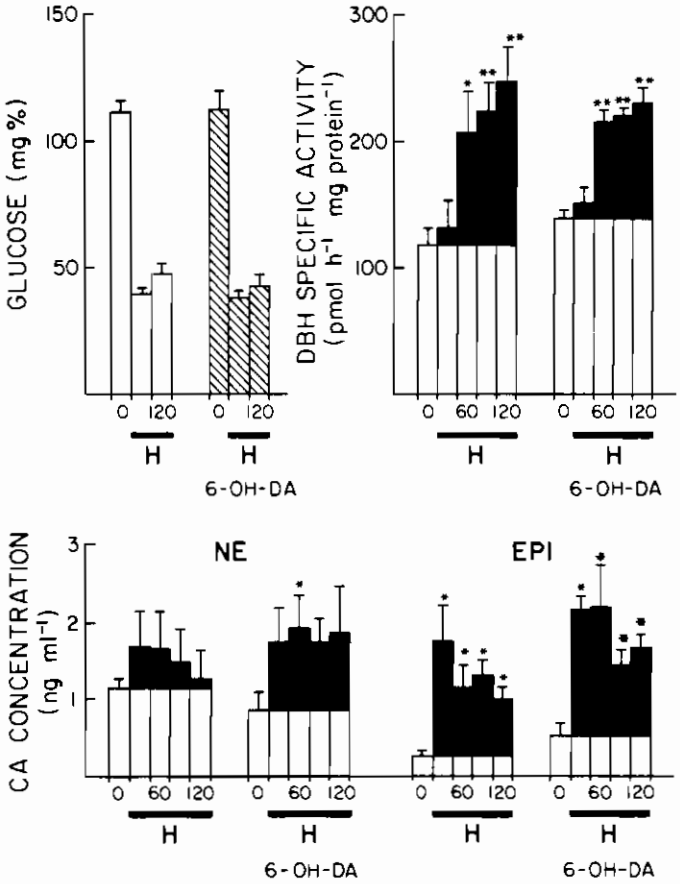

Fig. 2. Effects of IIH on plasma glucose, DBH and CA in awake cannulated control and 6-OHDA-treated guinea pigs. Both controls (untreated) (n = 6) and 6-OHDA-treated (n = 5) guinea pigs were subjected to IIH. Ordinates: plasma glucose concentration (mg/100 ml milligrams percent), plasma DBH specific activity (picomoles per hour per milligram of protein) and plasma CA concentration (nanograms per milliliter). Abscissa: time in minutes. The bars shown beneath indicate the time in minutes elapsed since the injection of insulin (5U/kg b.wt. i.a.) during which samples were collected. Shown are means ± S.E.M. Significantly different from basal values at * $P < .05$ and ** $P < .01$.

the CAs returned more rapidly to basal levels than DBH (fig. 1). The peak plasma levels of CAs reached during FI were around 12 ng/ml for NE and 5 ng/ml for EPI. Interestingly, during FI, the plasma concentration of NE was always significantly greater ($P < .01$) than basal levels; whereas for EPI, significant differences from prestress levels were only found at 10 and 20 min of stress (fig. 1).

Insulin injection to awake-cannulated guinea pigs induced a reduction in blood glucose (fig. 2). The basal plasma glucose levels averaged 112.8 ± 8.3 mg/100 ml and decreased to 38.6 ± 2.6 and 43.2 ± 5.1 mg/100 ml at 1 and 2 hr after the i.a. administration of insulin, respectively. The hypoglycemia was accompanied by a progressive increase in plasma DBH and a

rapid rise in the plasma concentration of EPI. The EPI concentration increased between 4 to 6 times above basal levels, whereas nonsignificant increases in plasma NE were observed (fig 2).

Although both FI and IIH increased the plasma levels of DBH and CAs, there were some qualitative and quantitative differences in the effects of both stresses (compare figs. 1 and 2). In fact, FI induced a 3-fold increase in plasma DBH, whereas a 1-fold increase was obtained with the hypoglycemic stress. In addition, the levels of plasma CAs reached in the restrained animals were much higher than those encountered in insulin-treated guinea pigs, and only with FI was there a significant increase in plasma NE. Furthermore, IIH appeared to induce a selective increase in plasma EPI, whereas FI evoked a much greater and sustained increase in plasma NE.

In additional control experiments, awake-cannulated guinea pigs were subjected to a similar sampling protocol, but were not exposed to either FI or IIH. No significant increases in the plasma levels of DBH, NE or EPI were observed in these animals (fig. 1).

Effects of 6-OHDA pretreatment on the changes in plasma DBH and CAs induced by FI or IIH in awake-cannulated guinea pigs. 6-OHDA was given i.a. in two doses of 23 mg/kg b.wt. each at 72 and 48 hr before subjecting the animals to FI or IIH. The degree of sympathetic denervation was assessed by measuring the content of CA and DBH in the heart, spleen and adrenal glands (table 2). With this treatment, there was an 80 to 90% reduction in the DBH and NE content of the spleen and heart; yet the EPI levels were not modified. As expected, 6-OHDA pretreatment failed to reduce the DBH, NE and EPI levels of adrenal glands; on the contrary, an increase in their contents was observed (table 2).

Chemical sympathectomy did not alter the basal plasma levels of DBH or EPI; however, those of NE were considerably reduced (table 1). With regard to the stress-induced changes in plasma CA and DBH, pretreatment with 6-OHDA diminished by 60 to 70% the peak increase and the rate of rise of plasma DBH induced by FI ($P < .001$) and prevented the rise in plasma CAs produced by this stress in control animals (fig. 1). The area under the curve for the increase in plasma DBH induced by FI was also reduced from 1541 ± 121 to 572 ± 42 pmol hr^{-1} mg^{-1} after 6-OHDA pretreatment ($P < .01$) (fig. 1).

Pretreatment with 6-OHDA failed to modify the hypoglycemic response and the increase in plasma EPI induced by insulin injection (fig. 2). Although the increase in plasma DBH induced by hypoglycemia was reduced by 25% in sympathectomized animals, this effect was not statistically significant. The area under the curve for plasma DBH was not calculated because the rate of fall of the enzyme activity was not followed. The levels of plasma NE were highly variable, and only at 1 hr was there a significant increase in the plasma concentration of this CA (fig. 2).

Rate of disappearance of plasma DBH: Effects of 6-OHDA pretreatment. Because FI induced larger increases in plasma DBH than IIH and because it was apparently easier to terminate the former stress procedure more rapidly, the rate of disappearance of plasma DBH from plasma was studied after subjecting the guinea pigs for 1 hr to FI ($n = 12$). Although 2 hr after releasing the animals from the securing ties the plasma DBH values were still elevated, nearly 60% of the FI-induced increase in plasma DBH disappeared from the 2nd to the 5th hr after stress cessation (figs. 1 and 2).

When the means of the experimental values were calculated and plotted on a semi-log graph paper, a biphasic rate of fall of plasma DBH was observed (fig. 3). In order to determine the kinetic parameters of the initial, more rapid declining line, the first order plot for the terminal portion of the second component was extrapolated to zero time and a different plot was made between the extrapolated line and the experimental points of the initial component (method of "feathering") (Gibaldi and Perrier, 1975). By employing this procedure, the following rate constant values were obtained: 0.69 hr^{-1} ($T_{1/2}$, 1:1 hr) and 0.09 hr^{-1} ($T_{1/2}$, 7.7 hr), for the first (k_α) and second (k_β) components, respectively (fig. 3). However, the individualized analysis of the data revealed that the biexponential decline in plasma DBH was not seen in all the animals studied. In fact, although the

TABLE 2

Effects of 6-OHDA on the splenic, cardiac and adrenal content of DBH, NE and EPI

The content of DBH and CA was determined in organs removed from control-untreated and 6-OHDA-treated guinea pigs which were not subjected to either FI or IIH. All the animals had been cannulated 72 hr before sacrifice and the organs were removed under sodium pentobarbital anesthesia. The mean weight of the organs for control animals were: 0.48 ± 0.05, 1.52 ± 0.15 and 0.32 ± 0.6 g for the spleen, heart and pair of adrenals, respectively; and in 6-OHDA-treated animals they were: 0.59 ± 0.11 spleen, 1.87 ± 0.18 heart and 0.45 ± 0.06 g for pair of adrenals. The results are expressed as total DBH or CA content per spleen, heart or pair of adrenals. Shown are mean values ± S.E.M. of *n* experiments.

Tissue	Treatment	DBH	% Change	NE	% Change	EPI	% Change
		nmol·hr^{-1}		μg		μg	
Spleen	Control ($n = 4$)	74 ± 29		1.1 ± 0.06		0.10 ± 0.04	
	6-OHDA ($n = 4$)	6 ± 2**	−92.2	0.21 ± 0.04**	−80.6	0.13 ± 0.02	+39.4
Heart	Control ($n = 8$)	1,550 ± 280		3.8 ± 1.0		0.54 ± 0.08	
	6-OHDA ($n = 8$)	117 ± 10**	−92.4	0.6 ± 0.07**	−84.2	0.56 ± 0.11	+4.1
Adrenal glands	Control ($n = 4$)	4,657 ± 527		11 ± 3		59 ± 16	
	6-OHDA ($n = 9$)	5,439 ± 489	+16.8	20 ± 2*	+77.9	106 ± 19	+80.4

* $P < .05$; ** $P < .01$.

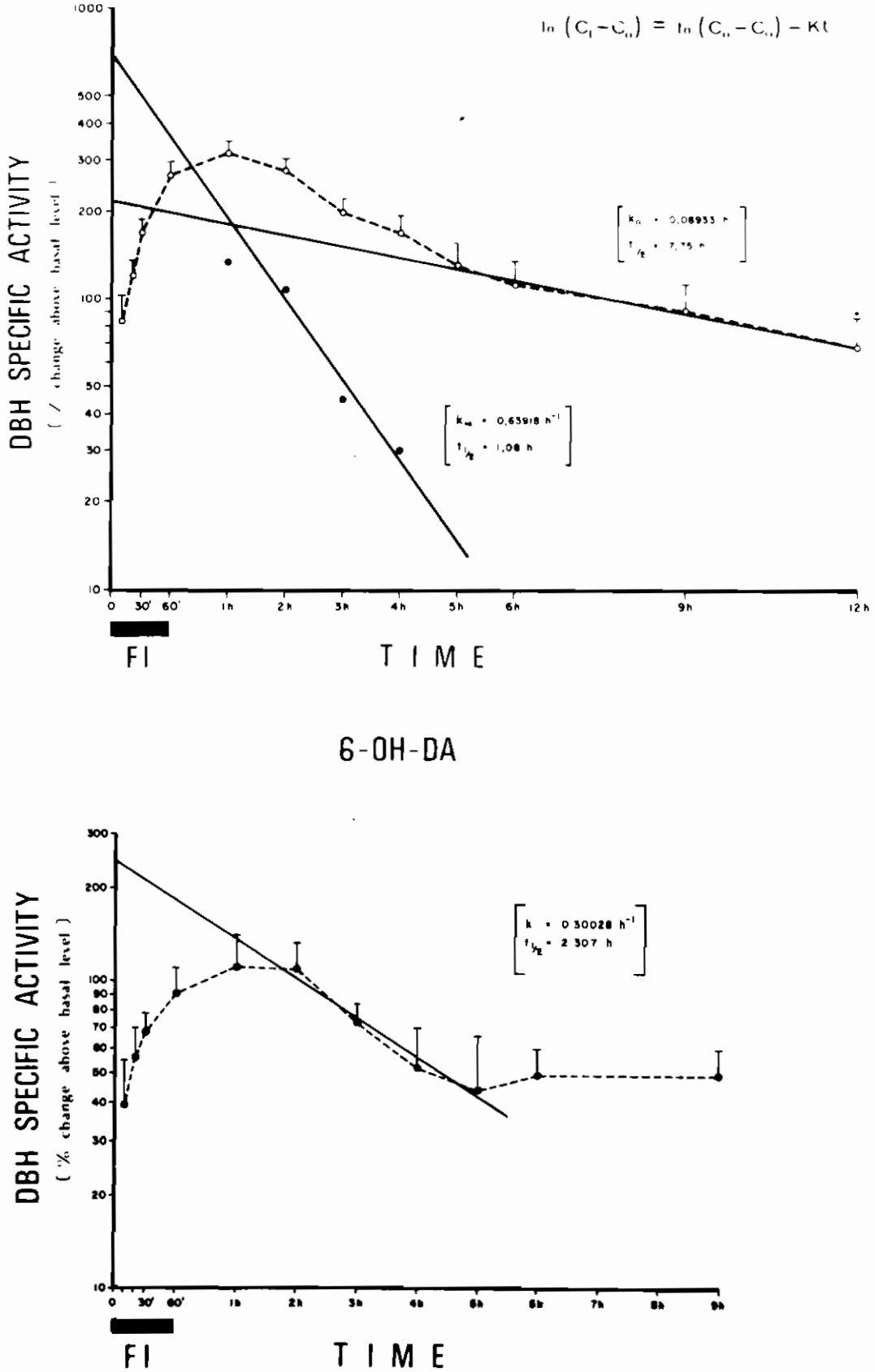

Fig. 3. Semilog plot of the changes in plasma **DBH** induced by FI in control and 6-OHDA-treated, awake cannulated guinea pigs. Normal (superior graph) or 6-OHDA-treated (inferior graph) guinea-pigs were exposed to FI for 1 hr and the decline in plasma DBH specific activity was followed up to 9 to 12 hr. The multiexponential curve was solved applying the feathering procedure (Gibaldi and Perrier, 1975). Within brackets are shown the kinetic constants obtained for each of the two lines. Ordinates: the specific activity of plasma DBH (picomoles per hour per milligram of protein) was expressed as the ln of the percentage of change above basal levels [ln (Ct-Co)]. *Abscissa:* time scale. The bar shown beneath indicates the duration of the FI period. Shown are mean values ± S.E.M.

first component (k_a) was present in all guinea pigs ($n = 12$), the slower compared appeared in only 60% of the animals. The rest of the guinea pigs (40%) showed no further significant decreases in plasma DBH within the 5th and 12th hr after stress termination. In the animals in which both components were clearly identified, the $T_{1/2}$ values averaged 1.3 and 4.0 hr, respectively. Interestingly, in those which showed only the initial, rapid component, the $T_{1/2}$ values were not significantly different from the rest of the guinea pigs. For the 12 animals studied, the k_a and $T_{1/2a}$ averaged 0.5 ± 0.08 hr^{-1} and 1.65 hr, respectively.

In the 6-OHDA-treated animals ($n = 6$), only a monophasic rate of decline was seen, inasmuch as no significant decrease in plasma DBH activity was observed within the 5th and 9th hr after stress cessation (fig. 3). The rate constant which describes the mean decline of plasma DBH in the 6-OHDA-treated guinea pigs was 0.3 hr^{-1} ($T_{1/2}$, 2.3 hr) (fig. 3). When the kinetic parameters were calculated for each of the animals and then statistically processed, they averaged 0.48 ± 0.05 hr^{-1} (k) and 1.55 hr ($T_{1/2}$). No significant differences were observed between controls and 6-OHDA-treated guinea pigs in the first order rate constants that describe the initial component for the disappearance of plasma DBH.

Discussion

Increases in plasma DBH evoked by acute stresses have been found to be dependent on the animal species studied, including man (Cubeddu, 1980). Although, marked species differences in the ratio of tissue releasable DBH to total circulating DBH have been reported (Barbella *et al.*, 1978; Cubeddu *et al.*, 1979), little is known with regard to the rate of elimination of the enzyme from plasma (Weinshilboum, 1978). On this issue, Roffman *et al.* (1973) followed the increase and subsequent decline to basal levels of rat serum DBH induced by a single swim stress of 2 hr. A similar approach was taken in the present study. In addition, Geyer *et al.* (1977) injected purified bovine or human DBH into rats and observed an initial rapid fall of enzyme activity during the first 5 hr, with $T_{1/2}$ values similar to those obtained by Roffman *et al.* (1973) and to those observed by us in guinea pigs (present study). Interestingly enough, calculations on the rate of fall of plasma DBH revealed that nearly 65% of the increase in enzymatic activity induced by stress or i.v. enzyme administration disappears by 4 hr, both in rats and guinea pigs. Consequently, it seems that the initial rapid kinetics play a major role in determining most of the decline in plasma DBH after short-term acute stresses and are probably related to the distribution of the enzyme in extravascular spaces (Rush and Geffen, 1972; Geyer *et al.*, 1977). The finding that guinea pigs and rats have similar values for the first order rate constants of "distribution" for plasma DBH indicates that the differences in the response of plasma DBH to acute stresses cannot be ascribed to differences in the rate of disappearance of the enzyme from plasma.

In other studies, a much longer $T_{1/2}$ component (days) has been found after the initial rapid kinetics (Geyer *et al.* 1977; Grzanna and Coyle, 1977, 1978). The failure to observe this very late component in our study could be due to the relatively small changes in plasma DBH induced by a single acute stress when compared to those produced by the injection of purified exogenous DBH (Geyer *et al.*, 1977). However, if such a component exists, its contribution to the total disappearance of plasma DBH under our experimental conditions would be negligible; in fact, it could only account for less than 3% of the total decline that occurred during the first 4 hr after stress termination. It is apparent from these findings that the redistribution processes seem to be the most important in reducing the augmented plasma DBH levels induced by short-term, acute stresses.

Although the magnitude of the increase in plasma DBH induced by FI was reduced in 6-OHDA-treated animals, a very rapid distribution for the kinetics of distribution of plasma DBH component was also found in the sympathectomized guinea pigs. Interestingly, similar values were obtained in controls and 6-OHDA-treated animals. These results indicate that the initial rapid phase of disappearance of DBH from plasma does not depend on the integrity of the noradrenergic nerves. The lack of the second component for the fall in plasma DBH observed in the 6-OHDA-treated animals could suggest that after its distribution the enzyme enters or binds to the sympathetic postganglionic nerves. However, the fact that 40% of the control untreated animals did not show a second component, speaks against such a possibility. In addition, the failure in observing a slower component could have been due to an insufficient sensitivity of the DBH assay in detecting very small changes in plasma DBH.

Although it is well accepted that an increase in plasma EPI reflects a greater activity of the adrenal medulla (Watts and Bragg, 1957; Kvetnansky and Kopin, 1978) and that plasma NE could derive from sympathetic nerves or adrenal glands (Kvetnansky *et al.*, 1978), the origin and significance of the increases in plasma DBH evoked by different stresses is not well known. Until recently, acute stress-induced increases in plasma DBH were ascribed solely to activation of sympathetic nerves. Studies by Cubeddu *et al.* (1977) and Pinardi *et al.* (1979) first demonstrated that stresses that activate the secretion of the adrenal medulla could lead to increases in plasma DBH. These initial observations have been confirmed in subsequent studies (Arnaiz *et al.*, 1978, 1980; Cubeddu *et al.*, 1979; present study). In the present work it was observed that hypoglycemia induced an increase in plasma DBH and a selective rise in plasma EPI. Interestingly, sympathectomized guinea pigs showed increases in plasma DBH and EPI similar to those of intact animals when exposed to a similar degree of hypoglycemia. These results suggest that hypoglycemia elicits a preferential release of CA and DBH from the adrenal glands. On the other hand, FI induced a much greater increase in plasma NE than EPI and chemical sympathectomy markedly diminished the increases in plasma CA and DBH in response to this stress. It is thus apparent that a preferential activation of noradrenergic postganglionic nerve fibers or of splanchnic nerves could be elicited depending on the stress employed.

In the rat, different stresses that increase the firing of the splanchnic nerves do not augment plasma DBH, even if accompanied by large increases in plasma EPI (see Cubeddu 1980, for review). The ratio, adrenal soluble DBH/circulating DBH, in the rat is 125 times lower than in the guinea pig, an animal in which large increases in plasma DBH occur when exposed to stress (Arnaiz *et al.*, 1978; 1980; Cubeddu *et al.*, 1979). Therefore, it has been proposed that the ratio content of adrenal soluble DBH/total amount of circulating enzyme is the important factor in determining the magnitude of the increases in plasma DBH produced by stresses that increase the discharge of the adrenal medulla (Cubeddu *et al.*, 1979). Because of the obvious difficulties existing in determining the total soluble DBH in sympathetic nerves, a similar ratio has not been obtained for the sympathetic system. However, based on the marked increases in plasma DBH elicited by FI in guinea pigs

(present study) compared to the very small changes produced by a similar stress in the rat (Weinshilboum *et al.*, 1977), one might suggest that guinea pigs should also have a much greater ratio, soluble DBH in sympathetic nerve terminals to circulating DBH, than rats. In conclusion, the different existing relationships between the contents of "releasable" DBH in sympathetic nerves and adrenal glands and the pool of circulating DBH seem to account for the variable species-dependent response of plasma DBH to acute stresses because differences in the rate of elimination of the enzyme from plasma do not appear to play an important role (present study).

References

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

ARNAIZ, J. M., GARCIA, A. G., HORGA, J. F., PASCUAL, R. AND SANCHEZ-GARCIA, P.: Origin of guinea-pig plasma dopamine-beta-hydroxylase. Br. J. Pharmacol. **69:** 41–48, 1980.

BARRELLA, Y., MARRERO, A., ISRAEL, A., TRIFARO, J. AND CUBEDDU, L. X.: Circulating dopamine-beta-hydroxylase (DBH) and catecholamines (CA) in dog, rat and guinea pigs during hemorrhagic and hypoglycemic stress. *In* Abstracts of the 7th International Congress of Pharmacology, Paris, Abstract 2401, p. 770, 1978.

BARTHELMAI, W. AND CZOK, R.: Enzymatische Bestimmungen der Glucose in Blut, Liquor and Harn. Klin. Wochenschr. **40:** 585, 1962.

CUBEDDU, L. X.: Release of dopamine-beta-hydroxylase: Discrepancy with in vivo plasma measurements. *In* Modulation of Neurochemical Transmission, ed. by E. S. Vizi, pp. 137–153, Pergamon Press, New York, 1980.

CUREDDU, L. X., BARBELLA, Y. R., MARRERO, A., TRIFARO, J. AND ISRAEL, A.: Circulating pool and adrenal content of dopamine-β-Hydroxylase (DBH) in rats, guinea-pigs, dogs and humans: Their role in determining acute stress-induced changes in plasma enzyme levels. J. Pharmacol. Exp. Ther. **211:** 271–279, 1979.

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

DA PRADA, M. AND ZURCHER, R. G.: Simultaneous radioenzymatic determination of plasma and tissue adrenaline and dopamine within the fentomole range. Life Sci. **19:** 1161–1174, 1976.

GARCIA, A. G., PELAYO, F. AND SANCHEZ-GARCIA, P.: Changes in plasma dopamine-beta-hydroxylase activity induced by stimulation of the complete sympathetic outflow in the pitched rat. J. Physiol. (Lond.) **278:** 287–296, 1978.

GEYER, S. J., SCHANBERG, S. M. AND KIRSHNER, N.: Turnover of dopamine-beta-hydroxylase in rat blood and lymph. *In* Structure and Function of Monoamine Enzymes, ed. by E. Usdin; N. Weiner and M. B. H. Youdim, pp. 423–438, Marcel Dekker, Inc. New York, 1977.

GIBALDI, M. AND PERRIER, D.: Pharmacokinetics, Marcel Dekker, Inc., New York, 1975.

GRZANNA, R. AND COYLE, J. T.: Immunochemical studies on the turnover of rat serum dopamine-beta-hydroxylase. Mol. Pharmacol. **13:** 956–964, 1977.

GRZANNA, R. AND COYLE, J. T.: Absence of a relationship between sympathetic neuronal activity and turnover of serum dopamine-beta-hydroxylase. Naunyn-Schmiedeberg's Arch. Pharmacol. **302:** 231–236, 1978.

KVETNANSKY, R. AND KOPIN, I. J.: Regulation of plasma epinephrine (EPI), noradrenaline (NE) and dopamine (DA) levels in stressed rats. *In* Catecholamines: Basic and Clinic Frontiers, 4th International Catecholamines Symposium, Asilomar, Abstract 226, p. 59, 1978.

KVETNANSKY, R. AND MIKULAJ, L.: Adrenal and urinary catecholamines in rats during adaption to repeated immobilization stress. Endocrinology **87:** 738–743, 1970.

KVETNANSKY, R., SUN, C. L., LAKE, C. R., THOA, N., TORDA, T. AND LOPIN, I.: Effect of handling and forced immobilization on rat plasma levels of epinephrine, norepinephrine and dopamine-beta-hydroxylase. Endocrinology **103:** 1868–1874, 1978.

LOWRY, O. N., ROSEBROUGH, N., FARR, A. L. AND RANDALL, R. J.: Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193:** 265–275, 1951.

MOLINOFF, P. B., WEINSHILBOUM, R. AND AXELROD, J.: A sensitive enzymatic assay for dopamine-beta-hydroxylase. J. Pharmacol. Exp. Ther. **178:** 425–431, 1971.

MUELLER, R. A., THOENEN, H. AND AXELROD, J.: Adrenal tyrosine hydroxylase: Compensatory increase in activity after chemical sympathectomy. Science (Wash, DC) **163:** 468–469, 1969.

PINARDI, G, TALMACIU, R., SANTIAGO, E. AND CUBEDDU, L. X.: Contribution of adrenal medulla, spleen and lymph, to the plasma levels of dopamine-β-hydroxylase and catecholamines induced by hemorrhagic hypotension in dogs. J. Pharmacol. Exp. Ther. **209:** 176–184, 1979.

PLANZ, G., WEITHOLD, G., APPEL, E., BOHMER, D., PALM, D. AND GROBECKER, H.: Correlation between increased dopamine-beta-hydroxylase activity and catecholamine concentration in plasma: Determination of acute changes in sympathetic activity in man. Eur. J. Clin. Pharmacol. **8:** 181–188, 1975.

REID, J. L. AND KOPIN, I. J.: Significance of plasma dopamine-beta-hydroxylase as an index of sympathetic neuron function. Proc. Natl. Acad. Sci. U. S. A. **71:** 4342–4394, 1974.

REID, J. L. AND KOPIN, I. J.: The effects of ganglionic blockade, reserpine and vinblastine on plasma catecholamines and dopamine-β-hydroxylase in the rat. J. Pharmacol. Exp. Ther. **193:** 748–756, 1975.

ROFFMAN, M., FREEDMAN, L. S. AND GOLDSTEIN, M.: The effect of acute and chronic swim stress on dopamine-beta-hydroxylase activity. Life Sci. **12:** 369–376, 1973.

RUSH, R. A. AND GEFFEN, L. B.: Radioimmunoassay and clearance of circulating dopamine-beta-hydroxylase. Circ. Res. **31:** 444–452, 1972.

SNEDECOR, G. W. AND COCHRAN, W. G.: Statistical Methods, 6th ed., Iowa State University Press, Ames, 1969.

WATTS, D. T. AND BRAGG, A. D.: Blood epinephrine levels and automatic reinfusion of blood during hemorrhagic shock in dogs. Proc. Soc. Exp. Biol. Med. **96:** 609–612, 1957.

WEINSHILBOUM, R.: Serum dopamine-β-hydroxylase. Pharmacol. Rev. **30:** 133–136, 1978.

WEINSHILBOUM, R. M., KVETNANSKY, R., AXELROD, J. AND KOPIN, I. J.: Elevation of serum dopamine-beta-hydroxylase activity with forced immobilization. Nat. New Biol. **230:** 287–288, 1971.

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