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Circulating Pool and Adrenal Soluble 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[1]

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ABSTRACT

Cubeddu, L. X., Y. R. Barbella, A. Marrero, J. Trifaro and A. S. Israel: *Circulating pool and adrenal soluble 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.

Dogs and rats anesthetized with pentobarbital were subjected to an acute hemorrhagic-hypotensive stress. In both species, the reduction of blood pressure to 40 mm Hg for 60 min, produced a 5- to 8-fold increase in plasma epinephrine, whereas the plasma dopamine β-hydroxylase (DBH) concentration and its specific activity was augmented only in dogs (2.5 times above basal levels). Similarly anesthetized rats and guinea pigs were injected with saline or crystalline insulin (5 U/kg b.wt. s.c.). In both species, 2 hr after insulin injection, there was a 60% reduction in the blood glucose levels and a 5- to 10-fold increase in plasma epinephrine. The plasma DBH concentration and specific activity was augmented only in guinea pigs (4 times above basal levels). The basal plasma DBH concentrations were 21.9, 6.6 and 2.4 nmol/hr/ml, in rats, guinea pigs and dogs, respectively. The total plasma DBH activity (nanomoles per hour) was much greater in dogs (1843 ± 151) than in rats (240 ± 38) and guinea pigs (123 ± 19). The total DBH activity present in two adrenal glands was 36,424, 12,029 and 435 nmol/hr in dogs, guinea pigs and rats, respectively. Guinea pigs had the highest proportion (55%) of soluble DBH in chromaffin granules. In rats and dogs, only 25% of the enzyme was found in the soluble form. Therefore, the total soluble ("releasable") DBH (nanomoles per hour) in adrenal glands was: 99 ± 10 in rats, 6666 ± 701 in guinea pigs and 9979 ± 1941 in dogs. The following ratios of adrenal soluble DBH/total plasma DBH, were obtained: 0.5, 5 and 63, in rats, dogs and guinea pigs, respectively. The higher the ratio, the greater the possibility of seeing increases in plasma DBH during acute stresses which augment the adrenal medullary discharge. Plasma DBH was measured in 17 normotensive, apparently healthy subjects, and adrenal glands were obtained from recently autopsied bodies. The human plasma DBH concentration was 1108 ± 163 nmol/hr/ml and the total plasma DBH activity averaged 2812 μmol/hr. The total adrenal DBH activity was 226 ± 16 μmol/hr and 51.3 ± 4.2% of the enzyme was in the soluble form. Humans had the lowest ratio of adrenal soluble DBH/total plasma DBH (0.04). From these results one could predict that acute stresses which increase the activity of the adrenal medulla would induce marked changes in plasma DBH in guinea pigs, moderate changes in dogs, minimal changes in rats and none in humans.

The formation of the neurotransmitter norepinephrine (NE) from dopamine is catalyzed by the enzyme dopamine β-hydroxylase (DBH) (Kaufman and Friedman, 1965). This enzyme is localized in the catecholamine-containing vesicles of noradrenergic nerve terminals and in the chromaffin granules of the adrenal medulla. Part of the enzyme is membrane-bound (particulate) and the rest is enclosed (soluble) inside the storage particles; the latter is assumed to be the fraction released by exocytosis (Stjärne and Lishajko, 1967; Oka *et al.*, 1967; Viveros *et al.*, 1968; Smith *et al.*, 1970).

DBH activity has been reported in plasma of humans and animals (Weinshilboum and Axelrod, 1971a; Cubeddu *et al.*, 1977; Cubeddu, 1978; Arnaiz *et al.*, 1978). If plasma DBH represents a result of release of enzyme with catecholamines (CAs) from the adrenal glands and sympathetic nerves, its measurement might give a useful index of sympathoadrenal activity. In rats, chemical sympathectomy produced by 6-hydroxydopamine reduced by 30% the basal plasma DBH concentration, whereas bilateral adrenalectomy or adrenal demedullectomy was without effect. In addition, in rats, the elevation of serum DBH after immobilization was not prevented by removal of the adrenal glands (Weinshilboum and Axelrod, 1971b; Weinshilboum *et al.*, 1971a). However, hemorrhage in dogs induced

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a marked increase in plasma DBH and CAs. During this stress, a large output of both substances was recorded from the left adrenal gland, but not from the spleen (an organ with a rich sympathetic innervation). In addition, adrenalectomy abolished the rise in plasma DBH and CAs induced by the bleeding stress (Cubeddu *et al.*, 1977; Pinardi *et al.*, 1979). Contrary to previous observations, these results indicate that stresses which induce acute changes in the activity of the adrenal medulla could lead to an increase in plasma DBH activity.

In the present study, we evaluated whether the aforementioned discrepancies were related to the type of stress (immobilization *vs.* hemorrhage) or to the animal species employed (rat *vs.* dog). Therefore, we compared the effects of acute hemorrhagic hypotension on the circulating levels of DBH and CAs in rats and dogs. In addition, we studied the effects of acute hypoglycemia on the plasma levels of both substances in two rodents, rat and guinea pig. In the latter species when exposed to a high atmospheric CO_2 content large increases in plasma DBH had been reported (Arnaiz *et al.*, 1978). Changes in circulating levels of DBH due to acute stress are probably related to the amount of enzyme released from storage sites, the size of the plasma pool of DBH and the rate of degradation of the enzyme in plasma. In the present study, we have determined the total soluble adrenal DBH ("releasable DBH") and the plasma pool of enzyme in rats, guinea pigs and dogs. In addition, similar measurements were performed in humans, to evaluate the possible clinical significance of plasma DBH as an indicator of adrenal medullary activity.

Methods

Hemorrhagic Hypotension Protocol

Mongrel dogs of either sex weighing 8 to 22 kg and male rats (Sprague-Dawley) weighing 300 to 350 g were anesthetized with sodium pentobarbital (60 and 30 mg/kg b.wt., respectively). A polyethylene cannula placed in the femoral artery was used to monitor arterial blood pressure and to obtain samples for chemical determinations, before and during the bleeding period. Pressure was recorded with strain-gauge transducers (Statham) and direct-writing recorders. Mean pressures were obtained by electrical integration and the heart rate was recorded by the arterial pressure signal. A total of 500 U of heparin was injected through the arterial cannula to each rat and 500 U/kg b.wt. was given i.v. to each dog. After a 30-min stabilization period, hemorrhage was induced by allowing rapid bleeding into a plastic reservoir. The mean arterial blood pressure was rapidly reduced to 40 mm Hg and maintained at this level for 1 hr. The microhematocrit of each sample was determined by centrifuging blood collected in capillary tubes at 15,000 $\times$ *g* for 5 min. At the end of the experiment, the adrenal glands were removed and assayed for DBH and CAs.

Insulin-Induced Hypoglycemia

Male rats weighing 300 to 350 g and guinea pigs of either sex weighing 350 to 600 g were fasted for 24 hr and then anesthetized with sodium pentobarbital (60 and 30 mg/kg b.wt. i.p., respectively). Ten min later, the animals were injected with either crystalline insulin (5 U/kg b.wt. s.c.) or saline and sacrificed by decapitation 2 hr later. Only the first 1.5 ml of blood coming out from the trunk of the body was collected in heparinized tubes and assayed for DBH, CAs and blood glucose. At the end of the experiment, the adrenal glands were removed for CAs and DBH determinations.

Human Samples

Blood samples for DBH determinations were obtained by antebrachial venipuncture from normotensive healthy subjects and kept for 30 min in a supine resting position. Plasma was prepared and stored at −40°C until assay.

Adrenal glands were provided by the State Department of Legal Medicine, from subjects (two males, two females) who died in car accidents, with postmortem delays of less than 4 hr. Once provided, the glands were immediately weighed and cleaned and the adrenal medullae were dissected and assayed either for total DBH or for soluble and particulate chromaffin granule enzyme.

Chemical Methods

DBH determination. *Plasma DBH activity.* Heparinized blood samples were immediately centrifuged at 12,000 $\times$ *g* for 10 min at 2°C, and the plasma was stored at −40°C. Before the assay, the plasma was diluted with ice-cold distilled water. The following dilutions were made (v/v): rats (1:20), guinea pigs and dogs (1:12) and humans (1:200–1:500). Appropriate copper concentrations were used to obtain optimal enzyme activity (1–10 μM, final concentration). Each sample was assayed in triplicate. The variability between replicate assay was 5.6 ± 1.4% (n = 36).

Adrenal DBH activity. The total DBH activity in adrenal glands was determined as follows. The adrenal glands were rapidly dissected, weighed and homogenized (rats and guinea pigs 1:100, w/v; dogs 1:20, w/v; humans 1:4, w/v) with an ice-cold 5 mM Tris-HCl solution, pH 7.3, containing 0.2% Triton X-100. The homogenate was centrifuged at 27,000 $\times$ *g* for 10 min; the pellet thus obtained was resuspended in the above solution and centrifuged at the same speed for 10 min. The resultant supernatants were combined and assayed for DBH activity. Appropriate dilutions of the supernatant with ice-cold distilled water (50 μl of 1:2000 dilution for rats, guinea pigs, dogs and humans) were used for the assay and different copper concentrations (1–10 μM) were employed for optimal enzyme activity. Samples were assayed in triplicate and incubated for 10 min during the first step (DBH step) of the assay. The variability between replicate assays was 3.9 ± 0.8% (n = 16).

Chromaffin granules were prepared from adrenal glands obtained from rats, guinea pigs, dogs and humans, as described by Smith and Winkler (1967). Briefly, the glands were homogenized with a Teflon pestle in a glass homogenizer containing 0.3 M sucrose, 25 mM Tris-HCl, pH 7.3, 0.1 mM pargyline and 500 U/ml of catalase. The homogenate was centrifuged twice at 800 $\times$ *g* for 10 min, and the pellets were discarded. The supernatant was applied to a single step 1.6 M sucrose gradient (2-ml supernatant on 7 ml of 1.6 M sucrose, containing 25 mM Tirs-HCl, ph 7.3, 0.1 mM pargyline and 500 U/ml of catalase) and centrifuged at 140,000 $\times$ *g* for 2 hr. The pellet (chromaffin granules) was lysed by two cycles of freezing-thawing and hypotonic shock in 5 mM Tris-HCl, pH 7.3, containing 0.1 mM pargyline and 500 U/ml of catalase. The soluble DBH was measured in the supernatant after centrifugation (140,000 $\times$ *g* for 120 min). The pellet (membrane-bound DBH) was resuspended in the above solution containing in addition 0.2% Triton X-100. In all cases, appropriate dilutions and different $CuSO_4$ concentrations (1–15 μM) were employed for linearity and optimal DBH activity. Both the soluble and particulate enzymes were incubated for 10 min during the DBH step. In all cases, the activity of DBH was determined by the procedure of Molinoff *et al.* (1971) as described in detail by Cubeddu *et al.* (1977). The DBH activity was expressed in nanomoles or micromoles of octopamine formed per hr.

CA determinations. *Plasma CA.* Heparinized blood samples were immediately centrifuged at 12,000 $\times$ *g* for 10 min at −2°C. Twenty microliters of a pH 6.5 solution containing 0.16 M reduced glutathion and 0.31 M ethylene glycol tris (β-aminoethyl ether)-N,N'-tetraacetic acid (EGTA), was immediately added to each milliliter of plasma. Subsequently, the plasma was deproteinized by adding an equal volume of ice-cold 0.6 N $HClO_4$ and the proteins were precipitated by centrifugation at 12,000 $\times$ *g* for 10 min. The CAs were measured by a combination of the radioenzymatic procedures of Da Prada and Zürcher (1976), Weise and Kopin (1976) and Cuello (1978). Briefly, 100 μl of the deproteinized plasma was incubated for 1 hr in glass-stoppered tubes at 37°C with 100 μl of the following mixture: 0.1 mg of dithiothreitol, 50 μl of 2 M Tris-HCl buffer (pH 9.6), 20 μl of 25 mM $MgCl_2$, 25 μl of partially purified rat liver catechol O-methyl-transferase and 5 μl of [^{3}H]methyl-S-adenosyl-methionine (25 μCi, specific activity: 12 Ci/mmol). The reaction was terminated by placing the tubes in an ice-cold

bath and by addition of 150 μl of 1 M sodium borate buffer, pH 8. Subsequently, 50 μl of a freshly prepared 1.5% aqueous solution (w/v) of tetraphenylboron and 50 μl of a 0.01 N HCl solution containing 0.5 mg/ml of the cold O-methylated CA metabolites were added to each tube. The O-methylated products were extracted into 4 ml of diethyl ether by vortexing the tubes for 30 sec. The ether was transferred to a new set of tubes containing 50 μl of 0.1 N HCl and vortexed vigorously for 30 sec. The ether was discarded and the HCl was subsequently washed with 2 ml of *n*-butyl acetate. After the organic solvent was decanted, the aqueous acid phase was transferred to the origin of Whatman No. 1 chromatographic paper previously spotted with 10 μl of the "carrier solution" (metanephrine, normetanephrine and methoxytyramine, 4 mg each in 1 ml of 0.01 N HCl). The papers were then developed overnight by descending chromatography in 25% methylamine–ter-amyl alcohol (1:4, v/v). The spots were visualized by exposing the paper chromatograms under an ultraviolet lamp (254 nm). The spots were cut out from the paper and placed in counting vials. The radioactivity was eluted from the paper with 2.5 ml of ethanol–concentrated ammonia (100:22, v/v) and counted by liquid scintillation spectrometry after addition of 5 ml of Aquasol-2. Blanks averaged 436.7 ± 95.4 cpm for norepinephrine (NE); 503.2 ± 56.2 cpm for epinephrine (EPI) and 363 ± 111 cpm for dopamine. Double blank values were obtained with 150 pg of each amine. Cross-contamination was less than 3.5%. Catechol O-methyltransferase was purified from young rats (100–150 g) as described by Axelrod and Tomchick (1958).

Tissues CA. Adrenal glands were immediately homogenized and deproteinized in $HClO_4$ (0.4 M final concentration). After centrifugation at 12,000 × *g* for 10 min the supernatants were collected and stored at −40°C until assay. The CA were determined fluorometrically as described by Laverty and Taylor (1968). Standard curves from 25 to 200 ng of NE and EPI and appropriate internal standards were routinely employed in the calculations.

Other determinations. Plasma glucose was determined as described by Nelson (1944) and proteins by the method of Lowry *et al.* (1951), using bovine serum albumin as standard. In dogs, the initial circulating plasma volume was measured with T-1824 (Evans blue dye) as described by Gregersen *et al.* (1950). In humans, the plasma volume was calculated from 80 ml/kg b.wt. and the hematocrit (42 ± 0.5%) (Milnor, 1974) and in guinea pigs and rats as 31.3 and 39.4 ml/kg (Altman and Dittmer, 1971).

Statistical calculations were performed according to conventional procedures (Snedecor and Cochran, 1969).

Chemicals. The drugs used were obtained from the following sources: *l*-arterenol-HCl, *dl*-epinephrine, dopamine, S-adenosylmethionine chloride, ascorbic acid, fumaric acid, sodium acetate, catalase and trizma base from Sigma Chemical Company, St. Louis, MO; crystalline insulin was from Eli Lilly, Venezuela.

Results

Effects of hemorrhagic hypotension in rats and dogs. Pentobarbital-anesthetized rats were bled until a blood pressure of 40 mm Hg was reached and after 60 min the blood contained in the reservoir was reinfused to the animal. During the hypotensive periods, there was a decrease in heart rate and hematocrit when compared to control rats (cannulated-anesthetized) (fig. 1). The maximal bleeding was obtained at 60 min and was 30% of the initial circulating blood volume, whereas in controls the maximal hemorrhage (due to sample collection) was less than 10% of the initial blood volume (fig. 1). No changes in the plasma DBH concentration were observed during and after hemorrhagic hypotension. However, the plasma enzyme specific activity (nanomoles per hour per milligram of protein) was significantly ($P < .05$) reduced at 30 min (fig. 1). Reinfusion of

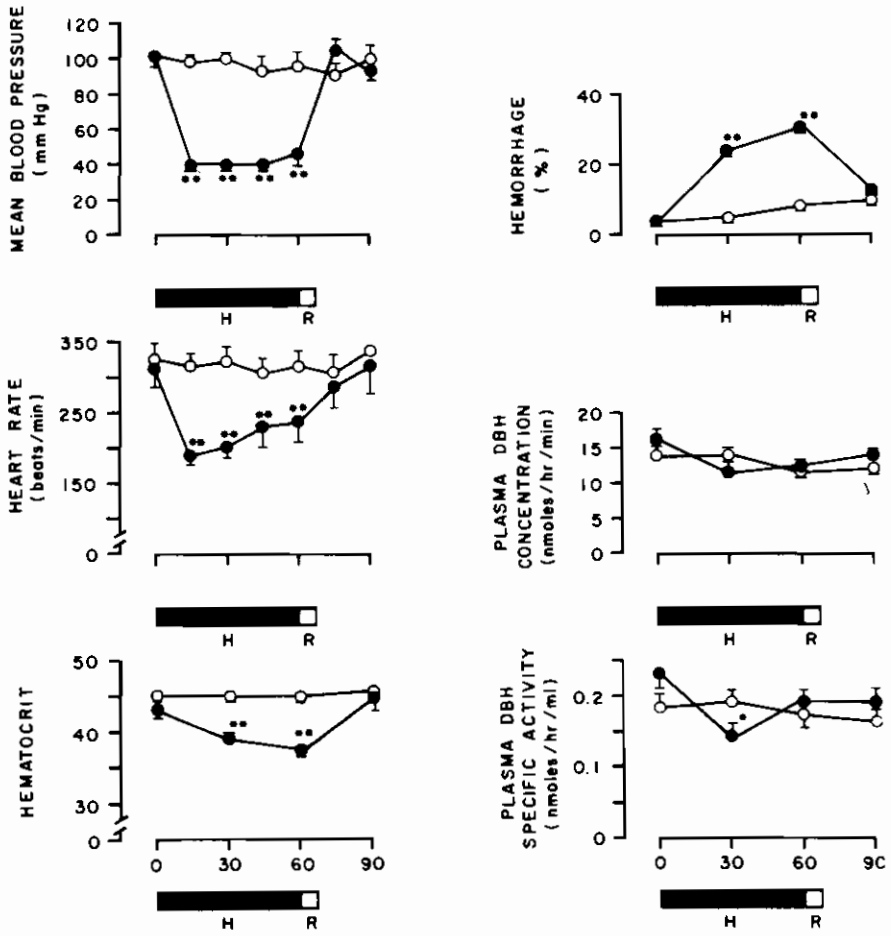

Fig. 1. Effects of hemorrhagic hypotension and blood reinfusion in rats, on mean arterial blood pressure, heart rate, hematocrit, percent hemorrhage, plasma DBH concentration and specific activity. Abscissa: time in minutes. The bars shown beneath indicate the duration of hemorrhagic hypotension [H] and blood reinfusion (R). ○, control animals (anesthetized, cannulated and in which blood samples were only taken for DBH determinations); ●, animals subjected to hemorrhagic hypotension and blood reinfusion. Results are mean values ± S.E.M. of six experiments per group; *$P < .05$ and **$P < .01$, significantly different from values obtained at similar times, in control rats.

the reservoir blood restored the hemodynamic parameters to control values and failed to modify the plasma DBH activity (fig. 1). In previous study from this laboratory (Cubeddu *et al.*, 1977), a marked and progressive increase in plasma DBH and CAs was observed in dogs subjected to hemorrhagic hypotension. With the purpose of investigating these differences, both CAs and DBH were simultaneously measured in plasma of rats and dogs subjected to the bleeding stress (fig. 2). In both species, hemorrhage induced a 5- to 8-fold increase in plasma CA levels, whereas the plasma DBH concentration was augmented only in dogs (2.5 times above control levels). In fact, in rats, either no change or a small decrease was observed (fig. 2). In both species, EPI was the main CA which increased. It accounted for 86 ± 4% and more than 95% of the total increase in plasma CAs, found in dogs and rats, respectively (fig. 2). The total plasma content of DBH (nanomoles per hr) in dogs was: 2521 ± 30 (before), 3005 ± 141 (at 30 min) and 3454 ± 265 (at 60 min after initiation of hemorrhage). In rats, the total plasma content of DBH averaged: 239 ± 27, 217 ± 28 and 197 ± 20 nmol/hr before and after 30 and 60 min of hemorrhage, respectively. In both species, there was an increase in the total plasma content of CAs. In dogs, it increased from 803 ± 208 ng (before) to 4008 ± 890 ng at 60 min of hemorrhagic hypotension. In rats, the basal plasma content of EPI was 14 ± 2 ng and increased to 59 ± 8 ng after 60 min of hemorrhage initiation.

Effects of hypoglycemic stress on the plasma concentration of glucose, CAs and DBH, in rats and guinea pigs. The effects of hypoglycemia, a stress known to increase the activity of adrenal medulla, was studied in two rodents, rats and guinea pigs. The animals were anesthetized and sacrificed by decapitation. As previously shown in rats (Popper *et al.*, 1977), there was a marked increase in the CA concentration in sequential samples of blood collected from the trunk of the body of awake or anesthetized rats and guinea pigs (fig. 3). However, no change was observed in the plasma concentration of DBH (fig. 3). The CA content of the first stream of blood coming out from the severed neck was much lower in pentobarbital-anesthetized than in awake decapitated animals (legend to fig. 3). The values obtained from decapitated anesthetized animals compare quite well with those reported in chronically cannulated animals (Popper *et al.*, 1977; Roizen *et al.*, 1978). Therefore, the initial stream of blood coming out from the trunk of the body of anesthetized animals was used for CA, DBH and glucose determinations.

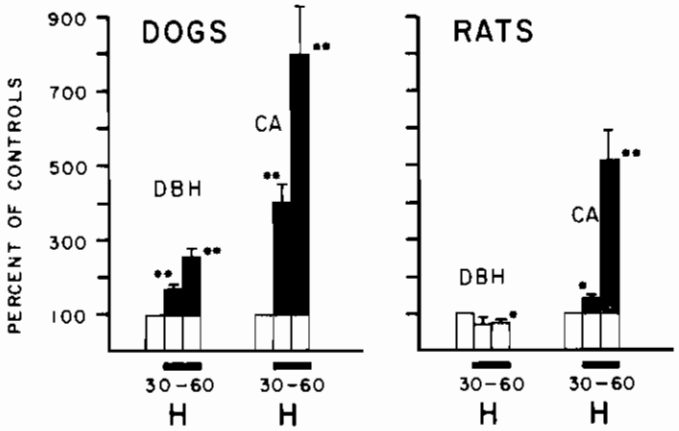

Fig. 2. Hemorrhage-induced changes in plasma DBH and CAs in dogs and rats. Pentobarbital-anesthetized dogs and rats were subjected to a similar hemorrhagic hypotension protocol. Blood samples were collected before and at 30 and 60 min of the hypotensive period. The prehemorrhage CA concentrations were: 0.99 ± 0.26 and 1.15 ± 0.3 ng/ml in rats and dogs, respectively. The prehemorrhage DBH concentration was: 16.5 ± 1.4 nmol/hr/ml in rats and 2.5 ± 0.2 nmol/hr/ml in dogs. Ordinates: changes in plasma DBH and CA concentrations expressed as percentages of control (prehemorrhage) values. Abscissa: the bars shown beneath indicate the samples taken during the period of hemorrhagic hypotension (H). Results are mean values ± S.E.M. of at least four experiments per group; $^{*}P < .05$ and $^{**}P < .001$, significantly different from values obtained in prehemorrhage samples.

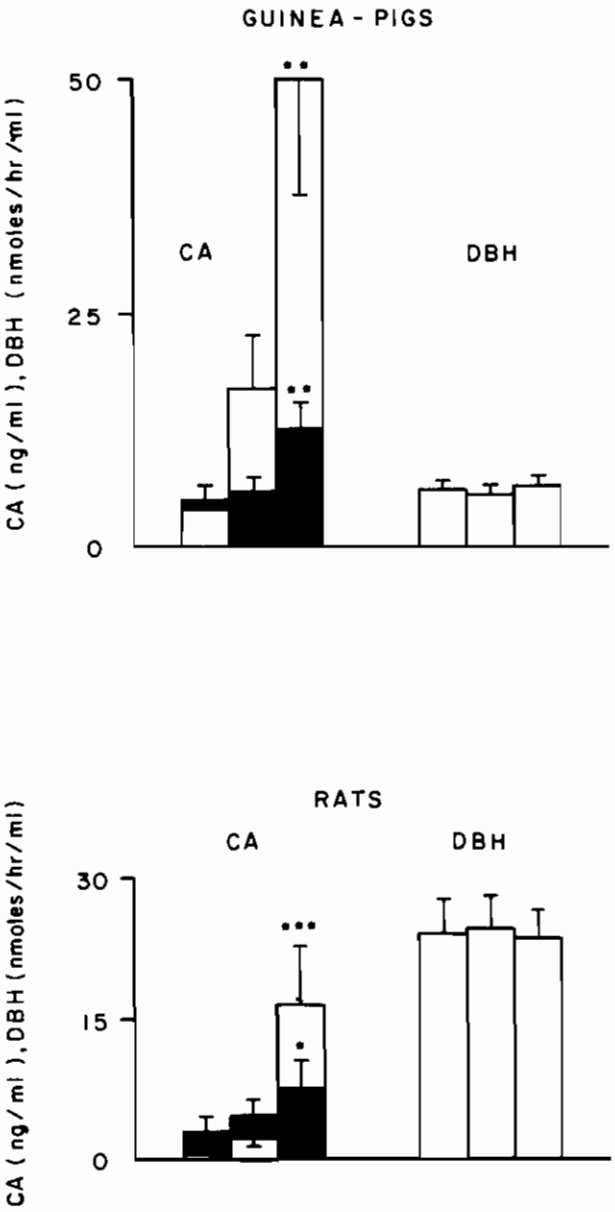

Fig. 3. Decapitation-induced changes in plasma CAs and DBH in guinea pigs and rats. Sequential samples of blood were collected from the trunk of the body of pentobarbital-anesthetized animals and assayed for DBH and CAs (EPI and NE). Ordinates: plasma concentrations of CAs (nanograms per milliliter) and DBH (nanomoles per hr per milliliter). The concentrations of CAs and DBH present in the first 1.5 ml of blood collected from the severed trunk, in awake animals was: in rats: NE: 6.8 ± 1.8, EPI: 5.2 ± 2.9 and DBH: 19.7 ± 0.5; in guinea pigs: NE: 10.1 ± 1.6, EPI: 14 ± 8 and DBH: 5.0 ± 0.2. Each bar represent mean ± S.E.M. of nine experiments. $^{*}P < .05$ and $^{**}P < .01$, significantly different from values obtained in the first 1.5 ml of blood.

Two hr after the s.c. injection of insulin (5 U/kg b.wt.), there was a 60% reduction in blood glucose and a 5- to 10-fold increase in the concentration of plasma EPI in rats and guinea pigs. However, no changes in either the plasma concentration or the specific activity of DBH were found in rats, whereas a marked increase (3-fold) was seen in guinea pigs (fig. 4). Plasma NE levels were not modified during insulin-induced hypoglycemia (fig. 4). To compare the intensity of the hemorrhagic and hypoglycemic stress in rats, the adrenal CA content was measured at the end of the experiments. In control anesthetized rats, the adrenal EPI content was 18.1 ± 1.6 μg/gland and that of NE was 1.2 ± 0.3 μg/gland. Hemorrhage induced a 66% depletion of adrenal EPI, whereas a 12% reduction was observed with the hypoglycemic stress. No significant changes in adrenal NE were observed with either type of stress.

Circulating pool and adrenal DBH activity in rats, guinea pigs and dogs. The circulating pool of DBH in rats, guinea pigs and dogs was estimated from the plasma enzyme concentration (nanomoles per hr per milliliter) and the plasma volume (milliliters) (table 1). The plasma DBH concentration and specific activity was: rats > guinea pigs > dogs. However, due to the larger blood volume of dogs the circulating pool of DBH (total plasma DBH) was much greater in dogs than in rodents (table 1).

Large differences were also observed in the weight of the adrenals and in the total DBH activity present in the glands of

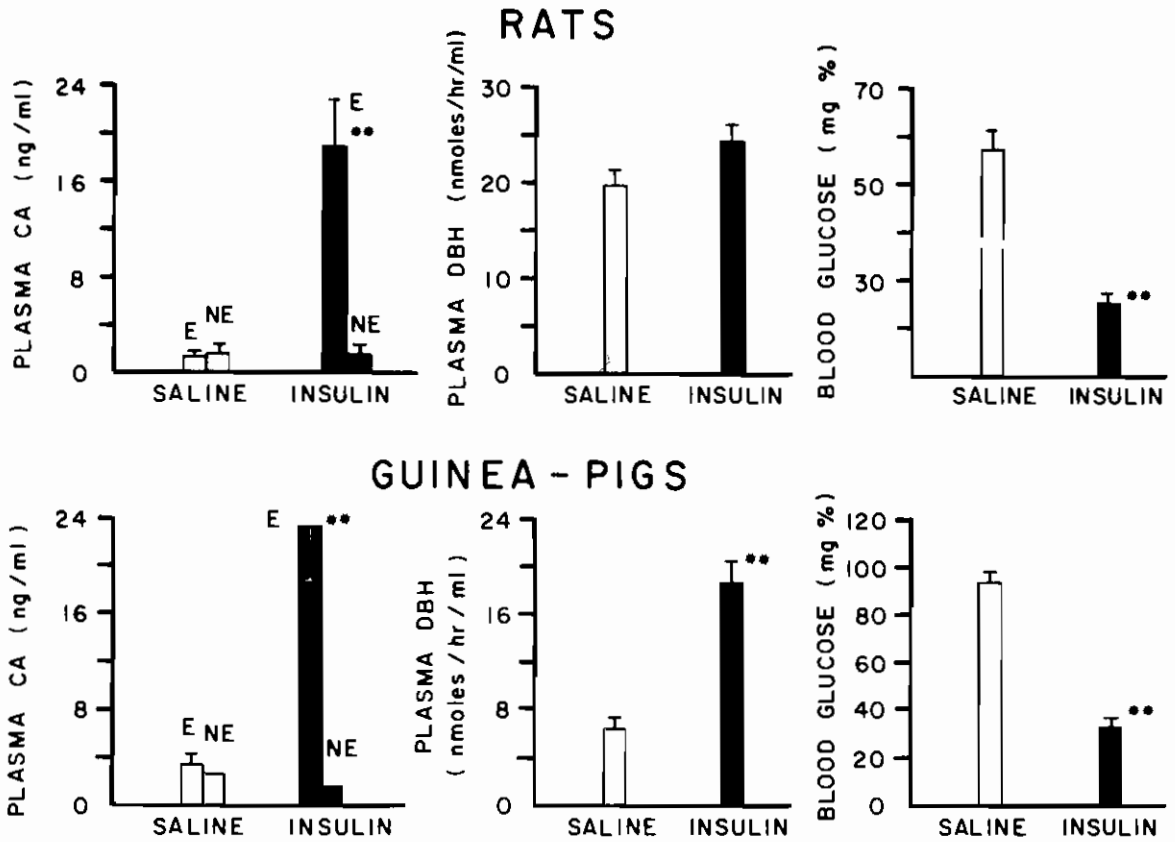

Fig. 4. Hypoglycemia-induced changes in plasma DBH, CAs and glucose in rats and guinea pigs. Twenty-four hr fasted pentobarbital-anesthetized rats and guinea pigs were injected with either saline (1 ml/kg b.wt. s.c.) or crystalline insulin (5 U/kg b.wt. s.c.). After 2 hr, the animals were decapitated. The first 1 to 2 ml of blood were collected and assayed for CAs, DBH and glucose levels. Ordinates: plasma concentrations of CAs (nanograms per milliliter), DBH (nanomoles per hr per milliliter) and blood glucose (milligrams per 100 ml). Each bar represents mean ± S.E.M. of nine experiments per group. **P < .001, statistically different from control (saline). The plasma protein concentration was (milligrams per milliliter): rats: saline = 75.5 ± 1.9, insulin = 73.1 ± 1.6; guinea pigs: saline = 58.5 ± 1.7, insulin = 54.9 ± 1.7. The plasma DBH specific activity was (nanomoles per hr per milligram of protein): rats: saline = 0.29 ± 0.04, insulin = 0.30 ± 0.02; guinea pigs: saline = 0.12 ± 0.01, insulin = 0.34 ± 0.05. **P < .001.

TABLE 1

Plasma DBH activity

The DBH and protein concentrations were determined in plasma obtained from blood collected by decapitation of rats and guinea pigs, and in dogs through a cannula placed in the femoral artery. In all cases, the animals were anesthetized with sodium pentobarbital. The circulating volume was estimated as described in "Methods." The total plasma DBH was calculated from the plasma DBH concentration and the plasma volume. Shown are mean ± S.E.M. of *n* experiments.

Species	DBH Conc.	Protein Conc.	Specific Activity	Plasma Volume	Total Plasma DBH
	nmol/hr/ml plasma	*mg/ml plasma*	*nmol/hr/mg protein*	*ml*	*nmol/hr*
Rat (*n* = 8)	21.9 ± 2.88	75.5 ± 1.87	0.290 ± 0.04	10.6 ± 0.5	239.9 ± 37.9
Guinea pig (*n* = 8)	6.6 ± 0.74**	58.5 ± 1.70**	0.120 ± 0.01**	17.9 ± 1.3**	123.3 ± 19.4**
Dog (*n* = 9)	2.4 ± 0.18	69.5 ± 5.2**	0.036 ± 0.002**	737 ± 60**	1843 ± 151**

** Significantly different at P < .001 from values obtained in rats.

rats, guinea pigs and dogs (table 2). Mainly due to the smaller size of the adrenal glands and partly to the lower enzyme activity per milligram of tissue, the total adrenal DBH in rats, was 30 and 80 times lower than that of guinea pigs and dogs, respectively (table 2). The adrenal chromaffin granule soluble DBH was determined. In guinea pigs, the proportion of soluble DBH was twice as great as that found in rats and dogs. The adrenal soluble enzyme content was calculated from the total adrenal DBH activity and the percentage of soluble DBH. In rats, the "total soluble DBH" was 70 to 100 times lower than in guinea pigs and dogs (table 2). The ratios of adrenal soluble DBH/circulating pool of DBH were calculated (table 3). In rats, the ratio gave a value lower than unity, and this was 10 times less than that of dogs and 130 times lower than in guinea pigs (table 3).

Pool of circulating DBH and adrenal content of DBH in humans. The plasma DBH concentration was determined in 17 normotensive, healthy subjects (nine males and eight females), with an age range of 22 to 64 years. The blood samples were obtained by venipuncture after the subjects rested for 30 min in a supine position. The plasma DBH concentration, the plasma volume and, thus, the circulating pool of DBH were very much greater in humans than in the animals studied (tables 1 and 4). The total circulating pool of enzyme was nearly 1500, 10,000 and 20,000 times greater in humans, than in dogs, rats and guinea pigs, respectively (tables 1 and 4). The DBH activity per milligram of human adrenal glands was not different from that observed in animals; thus, the greater total DBH activity found in human adrenal glands was due to the much greater size of the human glands (compare tables 2 and 4). In humans, the proportion of soluble DBH in chromaffin granules was similar to that found in guinea pigs (tables 2 and 4). However, humans had the lowest ratio soluble DBH/circulating pool of DBH (tables 3 and 4).

Since part of the differences observed in the circulating pool of DBH and in the total adrenal DBH activity could be due to differences in plasma volume and adrenal weight related to the size of the animal, the results were corrected by body weight (fig. 5). In fact, the adrenal DBH activity per kilogram body weight was nearly similar in rats, dogs and humans, whereas it was much greater in guinea pigs. In addition, the pool of circulating DBH in humans was between 50 and 400 times greater than that of the experimental animals studied. The data show the guinea pig, on one extreme, had a very large content of soluble adrenal DBH and a small circulating pool of enzyme. On the other hand, an extremely large pool of plasma DBH and a content of adrenal soluble DBH 25 times lower were found in humans (fig. 5).

Discussion

Measurements of plasma CAs and DBH have been employed to evaluate the sympathoadrenal discharge during acute stress situations. It is well accepted that an increase in plasma EPI reflects a greater activity of the adrenal medulla (Watts and

TABLE 3

Relationship between adrenal and plasma DBH

The total soluble adrenal DBH was calculated from the percentage of soluble enzyme in chromaffin granules and the total adrenal DBH activity (see tables 2 and 4). The total plasma DBH was estimated from the plasma DBH concentration and the plasma volume (see tables 1 and 4).

Species	Total Soluble Adrenal DBH / Total Plasma DBH
Rat	0.46
Guinea pig	62.90
Dog	5.14
Human	0.04

TABLE 4

Human DBH activity

Blood samples for DBH determinations were obtained by antebrachial venipuncture, in 17 healthy subjects, which had rested for 30 min in supine position. The circulating plasma volume was calculated from 80 ml/kg b.wt. and the hematocrit (42 ± 0.5%) (Milnor, 1974). The adrenal glands were provided by the Department of Legal Medicine from autopsied subjects with a postmortem delay of less than 4 hr. Chromaffin granules were prepared as described by Smith and Winkler (1967). The human plasma DBH activity was expressed in nanomoles per hr per milliliter; 60 nmol/hr/ml corresponds to 1 International Unit of plasma DBH (micromoles per min per liter) (Nagatsu and Udenfriend, 1972). Thus, the mean plasma DBH concentration averaged 18.5 ± 2.7 IU. The circulating pool of DBH and the total adrenal soluble DBH was calculated as described in the legend to table 3. Shown are mean ± S.E.M. of *n* experiments.

	Human Plasma DBH Activity (n = 17)
Plasma DBH concentration (nmol/hr/ml)	1108.0 ± 163
Plasma protein concentration (mg/ml)	67.0 ± 3.2
Specific activity of plasma DBH (nmol/hr/mg protein)	16.5 ± 2.7
Plasma weight (kg)	58.3 ± 3.1
Plasma volume (ml)	2592.0 ± 168
Total plasma DBH (μmol/hr)	2812.0 ± 339
	Human Adrenal DBH Activity (n = 4)
Total adrenal DBH activity (two glands) (μmol/hr)	226.0 ± 16
Adrenal DBH (μmol/hr/g tissue)	22.0 ± 2
Soluble DBH in chromaffin granules (% of total)	51.3 ± 4.2
Total soluble adrenal DBH (μmol/hr)	115.0 ± 10

TABLE 2

Adrenal DBH activity

DBH activity was measured in adrenal glands and in chromaffin granules prepared from adrenal glands of rats, guinea pigs and dogs. All of the animals were anesthetized with sodium pentobarbital. The total soluble DBH was calculated from the percentage of soluble enzyme in chromaffin granules and the total adrenal DBH activity. In the rat, guinea pig and dog, the medulla represented 56.8 ± 4.4, 62.4 ± 2.7 and 18.8 ± 0.5%, respectively, of the total adrenal weight. The adrenal DBH activity in medulla was 19 ± 2.7 nmol/hr/mg of tissue in rats, 81 ± 2.6 in guinea pigs and 112 ± 10 in dogs. Shown are mean values ± S.E.M. of *n* experiments.

Species	Adrenal Weight	Total Adrenal DBH (in Two Glands)	Adrenal DBH	% Soluble DBH (Chromaffin Granules)	Total Soluble DBH
	mg	*nmol/hr*	*nmol/hr/mg tissue*		*nmol/hr*
Rat (n = 8)	41 ± 2	435 ± 44	10.8 ± 1.6	23.3 ± 2.9	99 ± 10
Guinea pig (n = 8)	237 ± 27**	12029 ± 1280**	51.4 ± 1.9**	55.5 ± 4.2**	6666 ± 701**
Dog (n = 9)	1428 ± 127**	36424 ± 6737**	22.4 ± 3.8**	27.4 ± 3.1	9979 ± 1941**

** Significantly different at $P < .001$ from values obtained in rats.

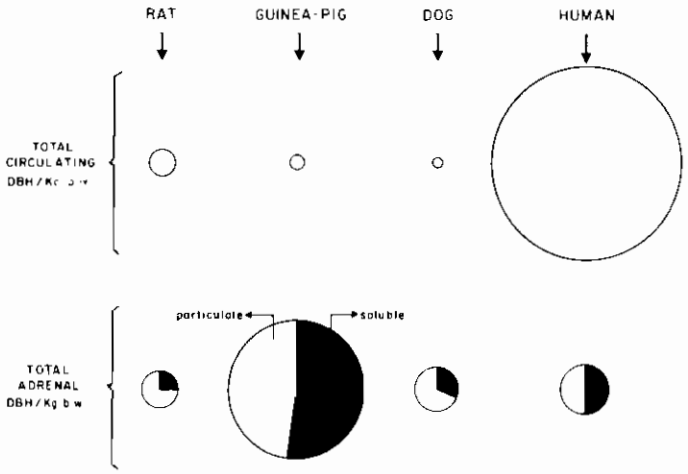

Fig. 5. Circulating pool and adrenal DBH activity in several animal species and man.

Bragg, 1957; Kvetnansky *et al.*, 1977; Kvetnansky and Kopin, 1978), whereas plasma NE could derive from either sympathetic nerves and/or the adrenal glands (Watts and Bragg, 1957; Feuerstein and Gutman, 1971; Reid and Kopin, 1975; Kvetnansky *et al.*, 1977). The origin and significance of plasma DBH activity is still a subject of controversy. Recently, a marked increase in plasma CAs (80% EPI; 20% NE) and DBH was observed in dogs subjected to hemorrhagic hypotension (Cubeddu *et al.*, 1977; present study), an effect prevented by bilateral adrenalectomy (Cubeddu *et al.*, 1977). In addition, in guinea pigs, hypoglycemia induced a marked and selective increase in circulating EPI, which was accompanied by a 3-fold elevation in plasma DBH (present study). These observations indicate that, at least in dogs and guinea pigs, acute increases in adrenal medullary activity can elevate the plasma DBH concentration.

In previous studies performed in rats, a small increase in the concentration of plasma DBH has been observed, after forced immobilization (Weinshilboum *et al.*, 1971b; Lamprecht *et al.*, 1973). The increase in plasma enzyme activity was not prevented by bilateral demedullectomy or adrenalectomy (Weinshilboum *et al.*, 1971b). However, it remains to be proved whether the immobilization-induced increase in plasma DBH is abolished by chemical sympathectomy, particularly, since EPI was the main CA which increased (90%) with this type of stress (Kvetnansky *et al.*, 1977; Kvetnansky and Kopin, 1978). In addition, it is known that stress-induced changes in plasma volume (hemoconcentration) and lymph flow, could modify the concentration of DBH in plasma, independently of a greater release of enzyme from its storage sites (Stone *et al.*, 1974; Pinardi *et al.*, 1979). Thus, plasma DBH activity should be routinely corrected for the plasma protein concentration, a factor which was not taken into account in that study (Weinshilboum *et al.*, 1971b). Thus, in the only case in which the source of the acute increase in plasma DBH has been unequivocally established, it was found that the enzyme derives from the adrenal gland (Cubeddu *et al.*, 1977; Pinardi *et al.*, 1979).

In the present study, large differences were observed in the response of plasma DBH to acute stresses in different animals. In fact, in rats and dogs subjected to a similar hemorrhagic hypotension protocol, there were large increases in circulating EPI, whereas the concentration of plasma DBH and its specific activity was only increased in dogs. Due to their vascular friability, hemorrhagic hypotension experiments could be not performed in guinea pigs. However, when rats and guinea pigs were subjected to insulin-induced hypoglycemia, DBH increased only in guinea pigs, whereas EPI was the CA that was markedly augmented in both rodents (present study). In addition, Arnaiz *et al.* (1978) reported an increase in plasma DBH in guinea pigs but not in rats, breathing a CO_2-enriched gas mixture; yet in both rodents, there was an increase in circulating NE (the authors did not measure plasma EPI), but in a previous study found that high CO_2 produces a complete depletion of adrenal CA (Dixon *et al.*, 1976). The results indicate that in the rat, acute stresses which increase the adrenal medullary discharge, as evidenced by increases in plasma EPI and/or a depletion of adrenal CA, fail to elevate the concentration and/or specific activity of plasma DBH.

Several factors could be involved in determining the different responses of plasma DBH in rats, guinea pigs and dogs to stresses which selectively increase the activity of the adrenal medulla, *i.e.*, hypoglycemia and hemorrhage (Watts and Bragg, 1957; Lewis, 1975), namely: 1) amount of enzyme released from the adrenal medulla, 2) size of the circulating pool of enzyme and 3) rate of degradation of plasma DBH. The amount of enzyme released from the chromaffin cells would depend on the duration and intensity of the stress and the total "releasable" (soluble) DBH. Although there is no direct proof that the enzyme which is released in an *in vitro* procedure such as freeze-thawing and hypotonic shock (soluble DBH) is the fraction of the DBH released by exocytosis, a relationship between the two has been suggested (Viveros *et al.*, 1968; Smith *et al.*, 1970). In the present study, differences due to stress were minimized by subjecting the experimental animals to similar stress models. The total soluble DBH was estimated from the proportion of soluble DBH in chromaffin granules and the total adrenal DBH activity. The pool of circulating enzyme was calculated from the plasma DBH concentration and plasma volume. The larger the pool of circulating enzyme, the greater should be the amount of enzyme released in order to see an acute increase in plasma DBH concentration. Thus, the higher the ratio of adrenal soluble DBH/pool of circulating DBH, the greater the possibility of obtaining elevations in plasma DBH following acute stresses that increase the discharge of the adrenal medulla. Interestingly, in the rat, the total adrenal DBH activity was only 75% greater than the pool of circulating enzyme. If 23% of the adrenal enzyme is in the soluble form releasable, the plasma enzyme pool would be twice as great as the total adrenal soluble DBH. Thus, even if all the soluble DBH is released at once, only a 40% increase in plasma DBH should be observed. Consequently, the low ratio of adrenal soluble DBH/plasma DBH found in rats probably accounts for the failure to observe increases in plasma DBH during acute stress situations which are accompanied by large increases in plasma EPI and/or depletion of adrenal CAs (Barbella *et al.*, 1978; Arnaiz *et al.*, 1978; Cubeddu, 1978; present study). If similar calculations are performed for dogs and guinea pigs, an increase in circulating DBH of 600 and 5500%, respectively, would be expected from a massive acute release of the soluble adrenal DBH. In fact, the larger reported stress-induced increases in circulating DBH have been observed in guinea pigs (Barbella *et al.*, 1978; Arnaiz *et al.*, 1978; present study). This result suggested that in this rodent, plasma DBH is a good indicator of acute changes in the activity of the adrenal medulla. This might be due to the very high ratio of adrenal soluble/total plasma DBH.

In rats and dogs, a similar percentage of soluble DBH was

found. However, the much greater adrenal enzyme content in dogs probably determines the fact that when subjected to similar stress procedures, a much greater amount of enzyme would be released in dogs than in rats. In fact, the ratio of adrenal soluble DBH/circulating DBH, was 10 times greater in dogs than in rats (present study).

When compared to the rat, dog and guinea pig, it was found that humans have the lowest ratio of adrenal soluble DBH/circulating DBH. In humans, the circulating pool of enzyme was 15 and 30 times greater than the total adrenal DBH and the soluble adrenal DBH content, respectively. These results clearly indicate that in humans acute stresses that augment the activity of the adrenal medulla should not increase the concentration of plasma DBH. Therefore, in humans, measurements of plasma DBH should not be of help in evaluating acute changes in adrenal medullary discharge.

According to the present study, increases in the plasma levels of DBH of chromaffin cell origin could be seen in large pheochromocytomas. Under these conditions the ratio of total and soluble chromaffin cells DBH/circulating DBH would be largely augmented. As predicted, marked increases in plasma DBH have been observed in patients with large pheochromocytomas. A relationship between the weight of the tumor (DBH content) and the basal DBH activity or the decrease in plasma DBH after surgical removal of the tumor has been described (Cubeddu, 1978; Kobayashi *et al.*, 1978; Valdivieso *et al.*, 1978). Obviously, increases in plasma DBH in pheochromocytoma could be related to the mechanism of CA secretion from the tumor, which may well vary from tumor to tumor. It is also possible that proteins (DBH) would be released from these tumors by a mechanism other than exocytosis. However, in small tumors where the ratio of total and soluble tumor DBH/circulating DBH is still low, no increases in the plasma enzyme level would occur independently of the mechanism of secretion of CA and proteins.

The reason for the large differences observed in basal plasma DBH activity between the laboratory animals and humans is not known. Even the low plasma DBH subjects (Weinshilboum *et al.*, 1975) have a much greater basal enzyme activity than rats, guinea pigs or dogs. In the present study, no relationships were observed among the total adrenal DBH content, the percentage of soluble DBH, the total adrenal soluble DBH and the pool of circulating enzyme. In fact, in spite of normalizing the data by kilograms body weight, the circulating pool of DBH in humans was 50 to 400 times greater than in the animals studied. On the other hand, a rather similar soluble adrenal DBH content per kilogram body weight was found in humans, dogs and rats, which was 25 times lower than that found in guinea pigs. From these findings, it is evident that the basal plasma DBH activity is not related to the adrenal enzyme content. In favor of this view are the findings of Weinshilboum *et al.* (1971a) and of Noth and Mulrow (1974), who reported that bilateral adrenalectomy failed to modify the basal plasma DBH concentration in rats and humans, respectively. Regarding the role of the sympathetic nerves to the basal plasma DBH activity, Weinshilboum and Axelrod (1971b) and Grzanna and Coyle (1978) reported that chemical sympathectomy with 6-hydroxydopamine or guanethidine produced only a moderate decrease (30%) in rat plasma DBH.

Although much has to be learned about the significance of the basal levels of plasma DBH, "*in vitro*" findings suggest that DBH may be released in the absence of nerve activity. In fact, in the cat and dog spleen, guinea pig vas deferens, heart and atria and in the perfused bovine and cat adrenals, a significant enzyme activity was present in perfusates and bathing media in the absence of neuronal depolarization. This "spontaneous loss" of DBH activity was not calcium dependent and thus possibly unrelated to exocytosis (Viveros *et al.*, 1968; De Potter *et al.*, 1972; Weinshilboum *et al.*, 1971b; Cubeddu *et al.*, 1974; Dixon *et al.*, 1976; Ackerly *et al.*, 1976; Langley and Weiner, 1978). Although there is no direct evidence that this continuous loss of enzyme occurs during *in vivo* conditions, recent findings of Grzanna and Coyle (1978) support such a possibility. In fact, after depletion of the plasma enzyme by treatment with anti-rat DBH antiserum, the rate of entrance of the enzyme into the blood stream was unrelated to the degree of sympathetic activity. Although these studies were performed in rats, a species in which the proportion of soluble DBH in sympathetic nerves and adrenal medulla is rather low (2–25%) (De Potter *et al.*, 1972; Brimijoin, 1974; Arnaiz *et al.*, 1978; present study), they support the view that basal plasma DBH could be a consequence of the release of DBH by a process different to that of exocytosis, *i.e.*, catabolism of vesicular components. However, increases in plasma DBH during acute stress procedures which increase the sympathoadrenal activity seem to be largely related to the ratio of total soluble DBH/circulating pool of DBH.

As mentioned before, differences in the rate of degradation of plasma DBH between species could determine the basal plasma enzyme levels and whether or not an increase in plasma DBH concentration would be seen during acute stresses. This possibility was not investigated in the present study. However, the much longer half-life of the enzyme than CAs could be responsible for the more rapid and greater increases in plasma CA levels than in DBH observed after acute stress procedures (Cubeddu *et al.*, 1977). This, together with differences in the size of the molecules (DBH *vs.* CA) thus in the rate of diffusion from storage sites to the circulation and (Viveros *et al.*, 1968; Cubeddu *et al.*, 1974; Pinardi *et al.*, 1979), could explain the absence of changes in plasma DBH concentration immediately after decapitation.

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