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THE EFFECTS OF GANGLIONIC BLOCKADE, RESERPINE AND VINBLASTINE ON PLASMA CATECHOLAMINES AND DOPAMINE-β-HYDROXYLASE IN THE RAT

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ABSTRACT

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In rats, chronic ganglionic blockade induced by repeated doses of chlorisondamine rapidly and profoundly lowered plasma norepinephrine, but plasma dopamine-β-hydroxylase (DBH) activity, even after 5 days treatment, was not significantly reduced. Long-term chlorisondamine treatment did not alter cardiac DBH or rapid axonal transport of DBH in sciatic nerve. Blockade of *alpha* adrenergic receptors by administration of repeated doses of phenoxybenzamine resulted in elevated levels of plasma catecholamines, but produced no change in plasma DBH. Chronic reserpine treatment (2.5 mg/kg on alternate days) increased plasma DBH after 2 and 5 days, whereas vinblastine (3 mg/kg) caused a progressive fall in enzyme activity in plasma over the same time period. It is concluded that plasma DBH activity does not closely parallel adrenergic function and neurotransmitter release in the rat. The level of DBH in plasma appears to reflect the rate of enzyme synthesis and axonal transport, It is likely that mechanisms other than stimulation-coupled exocytotic release determine levels of DBH activity in plasma.

Dopamine-β-hydroxylase (EC 1.14.2.1) (DBH) is the final enzyme in the biosynthetic pathway of norepinephrine.

The enzyme has been shown to be localized to intracellular vesicles of adrenergic neurones (de Potter *et al.*, 1970; Geffen and Livett, 1971). Release of the neurotransmitter during peripheral adrenergic nerve stimulation is accompanied by a proportional release of dopamine-β-hydroxylase and other constituents of the vesicle (Weinshilboum *et al.*, 1971a). Neurotransmitter release appears to be mediated by an exocytotic mechanism dependent on calcium and the integrity of the microtubular system (Thoa *et al.*, 1972). In the rat, serum DBH activity is not altered by adrenalectomy, but is reduced after intravenous administration of 6-hydroxydopamine, presumably as a result of destruction of peripheral sympathetic nerve endings (Weinshilboum and Axelrod, 1971a). It has been proposed that plasma DBH activity may reflect neurotransmitter release and provide an index of sympathetic neuronal activity. Several studies have supported such a relationship. Immobilization stress in rats (Weinshilboum *et al.*, 1971b;

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Lamprecht *et al.*, 1973) and cold pressor stress or exercise in man (Planz and Palm, 1972; Wooten and Cardon, 1973; Freedman *et al.*, 1973a; Frewin *et al.*, 1973) raise plasma DBH activity.

In a preliminary communication (Reid and Kopin, 1974), it was reported that impairment of adrenergic neurotransmission with bretylium or ganglionic blockade with chlorisondamine did not significantly change plasma DBH activity in rats whereas norepinephrine levels were reduced as expected. Furthermore, elevation of plasma catecholamines induced by administration of phenoxybenzamine was not attended by an increase in plasma DBH. In the present communication, we have extended these studies and shown that after prolonged periods of ganglionic blockade not only is plasma DBH activity not significantly altered, but cardiac levels of the enzyme and the rate of axonal transport of DBH in sciatic nerves are also unchanged. In contrast, reserpine causes a gradual increase in plasma DBH and vinblastine, which blocks axonal transport (Dahlstrom, 1971) and destroys sympathetic nerve endings (Hanbauer *et al.*, 1974), results in a progressive fall in plasma DBH activity.

Methods

Animals. Studies were performed with intact or adrenalectomized male Sprague-Dawley rats weighing 180 to 200 g (obtained from Zivic-Miller Laboratories, Allison Park, Pa.) which were housed six to eight per cage and fed on rat chow and water *ad libitum*.

Collection of blood. Cardiac blood was collected in heparin-treated tubes by percutaneous puncture of the heart 20 to 30 seconds after induction of light anesthesia by intravenous injection of sodium methohexital (10 mg/kg). Preliminary studies showed that plasma DBH and norepinephrine levels in blood collected by this procedure did not differ significantly from that in blood collected after decapitation. After centrifugation at 3000 rpm for 10 minutes at 4°C, the heparin-treated blood was divided into two aliquots: one to be assayed for DBH and the other for norepinephrine. In the aliquot to be assayed for norepinephrine, protein was precipitated by addition of concentrated perchloric acid (60%) to a final concentration of 0.1 N. Both aliquots were frozen and stored at −40°C for up to 14 days until assayed. There was no change in DBH activity or norepinephrine concentration when samples were stored under these conditions.

Assay of DBH in plasma. Plasma DBH activity was determined in plasma by the method of Weinshilboum and Axelrod (1971b) using 1.0 mM tyramine as substrate and 32 μM copper sulfate to obtain maximal activity. Samples were assayed in duplicate and activity was calculated using boiled plasma as blanks and octopamine standards. To correct for variations of phenylethanolamine N-methyltransferase (PNMT) activity in the second stage of the assay, samples of a standard stored pool of rat plasma were assayed for DBH activity along with each group of plasmas. Purified bovine adrenal DBH (Molinoff *et al.*, 1971) was used as an internal standard to show that there was no significant inhibition of DBH activity or interference with the PNMT stage of the assay in drug-treated groups of rats.

Assay of norepinephrine in plasma. Plasma norepinephrine was assayed by a sensitive radiometric enzymatic method (D. Henry, I. J. Kopin and V. K. Weise unpublished data). This method is a modification of earlier procedures (Saelens *et al.*, 1967; Iversen and Jarrott, 1970) and utilizes a partially purified preparation of PNMT (Molinoff *et al.*, 1971) and tritiated S-adenosylmethionine (4.5 mc/μmol) (New England Nuclear Corporation, Boston, Mass.). After centrifugation of 0.1 N perchloric acid homogenates at 10,000 rpm for 15 minutes, norepinephrine content of 0.05-ml aliquots of the supernatant is measured by conversion to ^{3}H-epinephrine. The tritiated product is adsorbed to alumina (Woelm neutral activity grade 1) and eluted with 0.1 N perchloric acid. After addition of unlabeled S-adenosylmethionine and precipitation of residual ^{3}H-S-adenosylmethionine with 25% phosphotungstic acid, tritiated epinephrine is extracted into 0.1% diethyldihexylphosphoric acid in toluene and the radioactivity in the organic phase is assayed by liquid scintillation spectrometry. Norepinephrine concentration is calculated using 0.1 N perchloric acid as blank and simultaneously run internal standards (0.5–1 ng of norepinephrine) in both pooled plasma from control and drug-treated animals. The assay is sensitive (0.04 ng of norepinephrine yields values twice blank) and specific for norepinephrine.

Cardiac DBH assay. For measurement of total cardiac DBH activity, the rats were killed by decapitation and the hearts were removed, weighed and homogenized in 25 volumes of ice-cold 0.005 M Tris buffer, pH 7.4, containing 0.1 v/v Triton X-100. After centrifugation at 15,000 × *g* for 10 minutes, DBH activity was deter-

mined in 0.1-ml aliquots of the supernatant using phenylethylamine as substrate in the presence of 97 μM copper sulfate as described by Molinoff *et al.* (1971). DBH activity is expressed as nanomoles of phenylethanolamine formed per hour per heart.

Sciatic nerve ligation. In order to examine axonal transport of DBH in sciatic nerves, rats were lightly anesthetized with ether and the nerve on one side was exposed by a dorsal incision in the thigh and ligated with silk thread (4-0) gauge). Eighteen hours later, animals were decapitated and the 1 cm of nerve immediately proximal to the ligature was removed. One centimeter of control unligated nerve was removed from the same region of the contralateral thigh. Nerve segments were homogenized in 1 ml of 0.005 M Tris buffer, pH 7.4, containing 0.2% w/v bovine serum albumin and 0.2% Triton X-100. DBH activity was measured in 0.2-ml aliquots using tyramine as substrate and 17 μM copper sulfate to inactivate inhibitors. DBH activity was expressed as nanomoles of octopamine formed per hour per centimeter of nerve and the percent increase in DBH activity after 18 hours calculated in the ligated nerve by comparison to the contralateral unligated side. Previous studies have shown that accumulation of DBH proximal to a ligation is linear over this time period (Wooten and Coyle, 1973).

Drugs. Chlorisondamine hydrochloride (Ecolid) and reserpine (Serpasil) were obtained from Ciba Pharmaceutical Company (Summit, N.J.). Phenoxybenzamine hydrochloride (Dibenzyline) from Smith Kline and French Laboratories (Philadelphia, Pa.) and vinblastine sulfate (Veban) from Eli Lilly and Company (Indianapolis, Ind.). All drugs were dissolved in 0.9% saline except reserpine which was used directly from commercially available ampules.

Results

Effect of chlorisondamine on plasma DBH activity. Within 1 hour after its administration, chlorisondamine (2 mg/kg i.p.) reduced plasma norepinephrine from 2.30 ± 0.24 ng/ml to 0.44 ± 0.09 ng/ml (mean ± S.E.M.). Repeated doses of the ganglionic blocking agent, administered at 8-hour intervals for 48 or 72 hours, maintained lowered plasma norepinephrine levels, confirming previous reports of the comparatively long duration of action of this ganglion blocking agent (Maxwell *et al.*, 1958), as rats were sacrificed 6 to 8 hours after the last dose of the drug. Although neurotransmitter release appeared to be markedly reduced and adrenergic function was imparied as evidenced by ptosis and lethargy during the intervals of drug action, there was no significant change in plasma DBH activity after 48 and 72 hours (table 1). Even after 5 days of treatment with chlorisondamine, plasma DBH remained at the same levels as in control, saline-treated animals.

Effect of chlorisondamine on rapid axonal transport and cardiac DBH activity. Axonal transport of DBH was investigated in control rats and groups of animals treated for 2 or 5 days with chlorisondamine. The sciatic nerve was ligated in mid-thigh on one side and the 1 cm immediately proximal to the ligature was removed after 18 hours with a similar length of unligated contralateral nerve from the same level serving as control. Ganglionic blockade did not alter significantly the DBH activity in unligated nerve segments. The activity in untreated controls was 1.56 ± 0.31 units /cm and in chlorisondamine rats was 1.37 ± 0.19 units/cm where 1 unit represents 1 nmol of octopamine formed per 1 hr of incubation. Eighteen hours after sciatic nerve ligation, there were increased levels of DBH in the nerve segment proximal to the ligature, presumably as a result of obstruction of axonal flow. The mean DBH activity the centimeter proximal to the ligation was similar in groups

TABLE 1

Plasma dopamine-β-hydroxylase activity after chlorisondamine

Treatment	Plasma DBH[a]
Controls (n = 17)	12.85 ± 0.64
Chlorisondamine[b] 48 hours (n = 14)	13.11 ± 0.94
Chlorisondamine[b] 72 hours (n = 5)	13.06 ± 1.87
Chlorisondamine[b] 5 days (n = 8)	12.83 ± 1.5

[a] Plasma DBH activity is expressed as nanomoles of octopamine per milliliter of plasma per 1 hour incubation.

[b] Chlorisondamine (2 mg/kg i.p.) was given three times daily to groups of rats (number of animals indicated in parentheses) for up to 5 days.

of animals treated with vehicle or chlorisondamine for 2 or 5 days (fig. 1). The ratio of DBH activity in the ligated and unligated nerves was 4.01 ± 0.68 in saline-treated controls and 4.72 ± 0.49 in chlorisondamine-treated animals. Mean transport rate of DBH in millimeters per hour was calculated from the rate of increase of enzyme after ligation, using formula:

$$\frac{(I-C)/t}{C/L} = \text{mm/hr}$$

where I is activity of enzyme in segment proximal to ligation, C is activity in the contralateral unligated nerves, t is the time (hours) from ligation to removal and L is the length of the segment in millimeters. The mean rate of axonal flow of DBH was calculated to be 1.91 mm/hr in controls and 1.98 mm/hr after chlorisondamine.

Total cardiac DBH activity did not appear to be altered by treatment with the ganglionic blocking agent for 2 or 5 days (table 2).

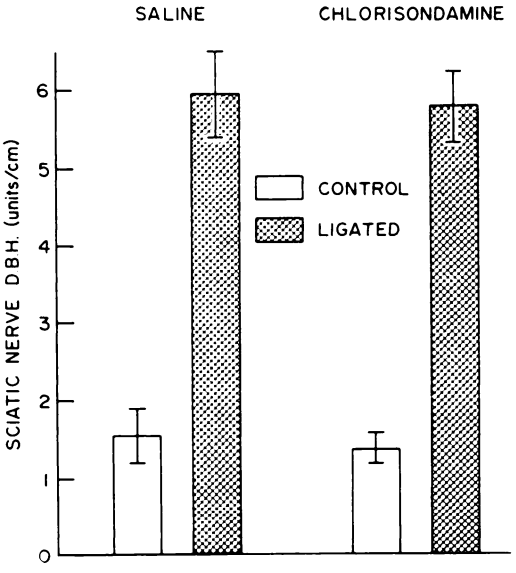

FIG. 1. Dopamine-β-hydroxlase activity in 1-cm segments of sciatic nerve. One unit is 1 nmol of octopamine formed per hr of incubation. Control unligated nerve segments are compared with 1-cm segments from the contralateral nerve immediately proximal to a ligature applied 18 hours before. Chlorisondamine (2 mg/kg i.p., 8 hourly) was given for 2 to 5 days (n = 12). Saline vehicle-treated animals served as controls. Results are mean values ± S.E.M. for groups of six to eight rats. **P < .01.

TABLE 2

Total cardiac dopamine-β-hydroxylase after chlorisondamine[a]

Treatment	Total Cardiac DBH Activity[b]
Controls	176.9 ± 16.2
Chlorisondamine, 2 days	183.2 ± 30.7
Chlorisondamine, 5 days	183.5 ± 15.3

[a] Groups of rats (n = 5) were given chlorisondamine (2 mg/kg i.p.) or saline vehicle every eight hours for 2 or 5 days.

[b] Mean (±S.E.M.) level of DBH activity is expressed as nanomoles of phenylethanolamine formed per heart per 1 hour incubation. Heart weights in treated (617 ± 26 mg) and untreated groups (655 ± 51 mg) were not significantly different.

Effect of phenoxybenzamine on plasma norepinephrine. Plasma norepinephrine rose rapidly after administration of phenoxybenzamine (10 mg/kg i.p.) reaching 9.29 ng/ml after 1 hour (table 3). The rise appeared to require sympathetic neuronal activity, since it was abolished by pretreatment with chlorisondamine. Although adrenal catecholamine release may have contributed to the rise, significant

TABLE 3

Acute effects of phenoxybenzamine on plasma norephinephrine[a]

	Without Phenoxybenzamine	1 Hr After Phenoxybenzamine
Control	1.70 ± 0.15	9.29 ± 1.20[b]
Chlorisondamine pretreated[c]	0.44 ± 0.09[d]	0.74 ± 0.15[d]
Adrenalectomized	1.58 ± 0.29	6.29 ± 1.26[b]

[a] Results are the mean (±S.E.M.) of plasma norepinephrine (nanograms per milliliter) before and 1 hour after treatment with phenoxybenzamine (10 mg/kg i.p.) for groups of six rats.

[b] P < .05 when phenoxybenzamine-treated groups are compared with their respective controls.

[c] Chlorisondamine (2 mg/kg i.p.) was given 1 hour before administration of the alpha receptor blocking agent.

[d] P < .05 when chlorisondamine-treated groups are compared with intact or adrenalectomized animals by 2-way analysis of variance

elevation of plasma norepinephrine still occurred in phenoxybenzamine-treated adrenalectomized animals (table 3). In intact rats, plasma norepinephrine remained high during repeated 8 hourly injections of phenoxybenzamine (fig. 2). Plasma DBH activity, which was unchanged after 8 hours, fell transiently, but not significantly, after 24 hours of treatment with the *alpha* receptor blocking agent and recovered to control levels at 48 and 72 hours.

Total cardiac DBH activity was significantly reduced ($P < .01$) by about one third at 24 hours and one-fourth at 48 hours after phenoxybenzamine. The mean activity of control hearts (nmol of phenylethanolamine/heart/hr) was 166.9 ± 6.7. After 24 and 48 hours, respectively, cardiac levels were 112.7 ± 7.6 and 123.6 ± 6.8 units.

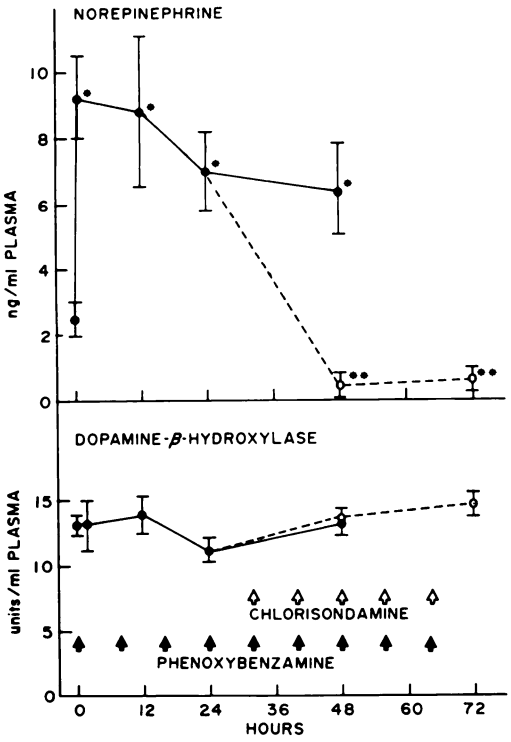

FIG. 2. Plasma norepinephrine nanograms per milliliter and DBH activity (Nanomoles of octopamine per milliliter per hour) in rats treated with phenoxybenzamine (10 mg/kg i.p., 8 hourly) (solid line) or phenoxybenzamine (10 mg/kg i.p., 8 hourly) for 24 hours followed by chlorisondamine (2 mg/kg i.p., 8 hourly) (dashed line). Results are mean values ± S.E.M. for groups of six to eight rats. *$P < .05$; **$P < .01$.

Another group of rats was given three doses of phenoxybenzamine at 8-hour intervals and then chlorisondamine (2 mg/kg) 8 hourly for 48 hours. Plasma norepinephrine was lowered while plasma DBH was maintained at base-line levels (fig. 2).

Effect of reserpine on plasma DBH and norepinehrine. In rats treated with reserpine (2.5 mg/kg i.p., on alternate days), plasma norepinephrine was reduced ($P < .01$) after 24 hours, 2 days and 5 days. Plasma DBH activity was unchanged at 24 hours but was significantly higher than control at 2 and 5 days (fig. 3).

Effect of vinblastine on plasma DBH and norepinephrine. Intravenous injection of vinblastine (3 mg/kg) caused a fall in plasma DBH activity which was significant ($P < .01$) at 5 days (fig. 4). At this time, DBH activity in plasma was about two-thirds that of saline-treated control rats. Plasma norepinephrine was unchanged 24 and 48 hours after vinblastine

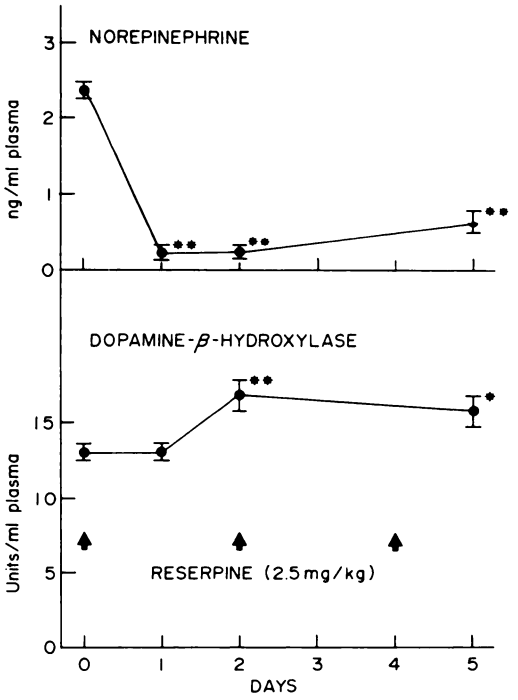

FIG. 3. Effect of reserpine (2.5 mg/kg i.p., on alternate days) on plasma norepinephrine (nanograms per milliliter) and plasma DBH activity (nanomoles of octopamine per milliliter per hour of rats. Results are mean values ± S.E.M. for groups of six to eight rats. *$P < .05$; **$P < .01$.

(fig. 4). Although higher levels of norepinephrine were measured after 5 days, the change was not significant.

Discussion

Prolonged ganglion blockade with chlorisondamine in doses which profoundly diminish sympathoadrenal function (Maxwell *et al.*, 1958) and markedly reduce circulating levels of norepinephrine did not lower plasma DBH activity. The absence of effect on plasma DBH was associated with an unchanged rate of axonal transport of the enzyme and no change in total DBH content of nerve endings in the heart. These results suggest that blockade of ganglionic neurotransmission did not alter the rate of synthesis of DBH in neuronal cell bodies, the transport of the enzyme along the axon or the turnover of the enzyme at the nerve ending.

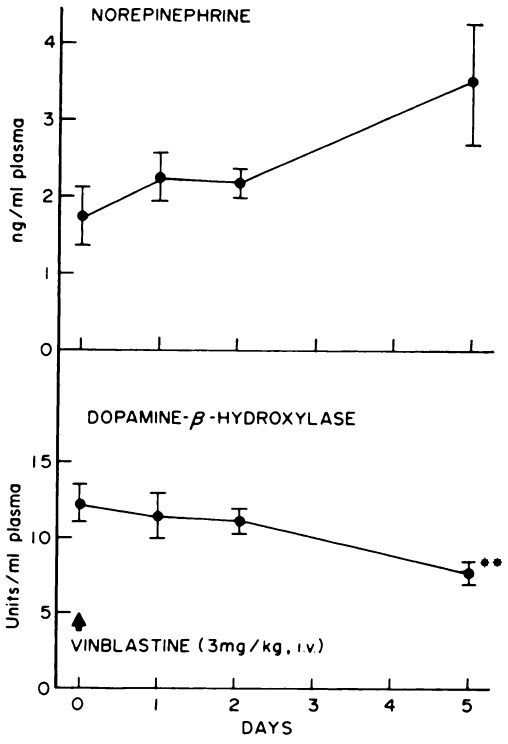

FIG. 4. Effect of vinblastine (3 mg/kg i.v.) on plasma norepinephrine (nanograms per milliliter) and plasma dopamine-β-hydroxylase (nanomoles of octopamine per milliliter per hour) of rats. Results are mean values ± S.E.M. for groups of six to eight rats. *P < .05; **P < .01.

Phenoxybenzamine, which raises plasma norepinephrine at least in part by reflex mechanisms dependent on ganglionic neurotransmission, caused a fall in DBH activity in cardiac nerve endings. This fall could reflect exhaustion of the supply of releasable DBH at the nerve endings. The rise of cardiac DBH levels towards normal after 48 hours may be due to continued or possibly enhanced synthesis, and replacement by axonal transport, of the enzyme.

Reserpine depletes neuronal monamine stores and modifies sympathetic neuron function by impairing amine incorporation into vesicles (Stjärne, 1964). This results in a lack of norepinephrine (NE) available for release from nerve endings with a fall in plasma NE. A compensatory increase in sympathetic activity presumably results. Since reserpine treatment does not interfere with stimulation-induced release of DBH from the guinea-pig vas deferens (Thoa *et al.*, 1975), a rise in plasma DBH might be expected. One day after reserpine administration, however, plasma DBH levels are not changed (fig. 3). DBH activity in sympathetic ganglia is elevated 1 to 2 days after reserpine and, 24 hours after the increase in the ganglia, DBH activity is increased in the heart (Molinoff *et al.*, 1972). Two and 5 days after reserpine, plasma DBH activity is also increased (fig. 3) suggesting that after reserpine treatment, release of DBH is enhanced only after the levels of DBH in the peripheral nerve endings are increased.

Vinblastine and related aklaloids have several actions on adrenergic neurons, including disruption of the microtubular system (Dahlstrom, 1971), blockade of axonal transport of enzymes (Wooten and Coyle, 1973) and a neurotoxic, 6-hydroxydopamine-like effect on nerve endings (Hanbauer *et al.*, 1974). Vinblastine has been reported previously to reduce DBH activity in the superior cervical ganglion and the heart (Hanbauer *et al.*, 1973). In the present study plasma DBH also fell progressively over a time course similar to that in the heart. The magnitude of the fall in plasma DBH after vinblastine is similar to that observed after intravenous administration of 6-hydroxydopamine (Weinshilboum and Axelrod, 1971a) which also destroys adrenergic nerve endings (Thoenen

and Tranzer, 1968). The lack of change in plasma NE may reflect compensatory increases in amine release from remaining neurons or enhanced adrenal medullary amine release (Hanbauer *et al.*, 1973).

The parallel changes in plasma DBH activity with changes in superior cervical ganglion (cell bodies) and heart (nerve endings) after reserpine and vinblastine, and the failure of ganglion blockade to influence the axonal transport, or cardiac or plasma levels of DBH indicate that although plasma DBH does not follow pharmacologically induced changes in sympathetic neuronal activity, it does appear to reflect the rate of synthesis of the enzyme in adrenergic cell bodies.

Rat plasma DBH activity was first proposed as a possible index of sympathetic function as it was not influenced by adrenalectomy, but fell after chemical sympathectomy with 6-hydroxydopamine (Weinshilboum and Axelrod, 1971a). Furthermore, there is a proportional release of DBH together with norepinephrine after stimulation of sympathetic neurones of *in vitro* preparations (Weinshilboum *et al.*, 1971a), suggesting that exocytosis in response to nerve stimulation was the mechanism of release of both amine and enzyme. This hypothesis was supported by reports of increases in plasma DBH after immobilization stress in rats (Weinshilboum *et al.*, 1971b; Lamprecht *et al.*, 1973) and several autonomic stresses in man (Wooten and Cardon, 1973; Freedman *et al.*, 1973b; Planz and Palm, 1972). Other groups have reported less consistent acute changes in plasma DBH after similar procedures (Wetterberg et *al.*, 1972; Mueller *et al.*, 1972; Frewin *et al.*, 1973; Horowitz *et al.*, 1973). Stone *et al.* (1974) did observe small, acute changes in plasma DBH after cold pressor stress in man but noted similar changes in other macromolecular constituents of plasma not generally believed to be associated with adrenergic function. These authors concluded that acute changes in plasma DBH are probably not related to neuronal DBH release.

Schanberg *et al.* (1974) proposed that over a longer time period plasma DBH might be indicative of sympathetic function and found a correlation between blood pressure, plasma DBH and urinary catecholamine excretion. Geffen *et al.* (1973) also noted a correlation of plasma DBH, measured by immunoassay, with plasma norepinephrine and blood pressure in hypertensive patients.

Geffen and Rush (1973) observed a poor correlation between measurements of DBH activity and immunoreactive DBH in plasma and suggested individual differences in the rate of clearance and degradation of the enzyme in plasma might compromise the usefulness of the enzymatic activity measurements and explain the absence of a correlation of plasma DBH activity with blood pressure reported by Horowitz *et al.* (1973). However, more recent studies using other sources of antigen have demonstrated a good correlation between DBH measured as enzymatically active and immunoreactive protein (Ebstein *et al.*, 1973) and support the validity of studies using DBH activity to assay circulating levels of the enzyme.

Measurements of plasma DBH in a wide range of clinical conditions known or suspected to be associated with autonomic dysfunction have failed to demonstrate consistent differences in DBH activity (Freedman *et al.*, 1973b).

Genetically determined factors appear to underlie the wide variation in plasma DBH in man (Weinshilboum *et al.*, 1973; Ross *et al.*, 1973). The genetic control of plasma DBH is probably multifactorial and may involve, in addition to differences in neuronal release, clearance and degradation of DBH, differences in the rate of enzyme synthesis in neuronal cell bodies in sympathetic ganglia.

Our observations in an inbred strain of rats suggest that the rate of enzyme synthesis determines the level of enzyme in the plasma and that over a long period of altered sympathetic activity, plasma levels do not closely parallel changes in neurotransmitter release. Although stimulus-coupled exocytotic release of norepinephrine is accompanied by release of small amounts of DBH, we suggest that the amount of DBH reaching the systemic circulation by this mechanism is transient and relatively small. The subcellular distribution of DBH in nerve endings indicates that only about 10% of the enzyme in the vesicles is freely soluble (Wooten, 1973). Most of the enzyme is bound to the vesicular membrane. The fate of this

form of DBH is not clearly understood. Apparently, DBH can leave nerve terminals by mechanisms not dependent on neuronal depolarization and transmitter efflux. DBH is released from *in vitro* preparations such as the isolated guinea-pig vas deferens in absence of nerve stimulation and even in the presence of colchicine or vinblastine which block exocytotic release of neurotransmitter (Thoa *et al.*, 1972).

Among the possible models of stimulus-independent DBH release which would be compatible with our observations are: 1) continuous spontaneous fusion of vesicles with the axonal membrane and slow release of bound DBH from the internal vesicular membrane now contiguous with the external axonal membrane; and 2) reformation of the vesicular membrane after release of the intravesicular contents and the intracellular return of the vesicle. The vesicle, which may or may not undergo cycles of fusion and release of contents, would ultimately be destroyed and the DBH associated with the membrane extruded from the cell by a mechanism not coupled to neuronal deplorization and /or partially destroyed in the nerve ending. The relative importance of release or destruction might vary among species of individuals. In circumstances where the DBH (or vesicular) turnover was increased, *e.g.*, after treatment with reserpine, more DBH would enter the circulation. Impairment of enzyme transport or disruption of nerve terminals, as after treatment with 6-hydroxydopamine or vinblastine, would diminish synthesis and release of the enzyme and cause a fall in plasma DBH.

Chlorisondamine, which does not alter axonal transport or tissue levels of enzyme, may not change the rate of turnover of DBH (or vesicles) in the terminals and although exocytotic release of DBH would be diminished, the total amount of DBH released would not differ significantly from that of untreated animals and plasma DBH activity would remain unchanged.

In conclusion, in the rat, plasma DBH activity after pharmacological modification of sympathetic neuron function did not parallel adrenergic activity or neurotransmitter release, but was more closely correlated to the rate of synthesis of the enzyme in cell bodies. Although plasma DBH activity in an individual may be influenced by changes in autonomic function, the principal factors regulating plasma levels of DBH are genetic and other influences which modifiy the rates of synthesis and degradation of the enzyme.

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