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Dopamine-β-hydroxylase: Regulation of Its Synthesis and Release from Nerve Terminals

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Properties of Dopamine-β-Hydroxylase

DOPAMINE-β-HYDROXYLASE (DBH) plays an important role in neurotransmission in the sympathetic nerve because of its localization and the reaction it catalyzes. This enzyme hydroxylates dopamine on the *beta* carbon to form noradrenaline. It has been purified and has been shown to have a molecular weight of 290,000 (9), and contains about 2 μmoles of Cu^{++} per μmole of enzyme (10, 11). The enzymatic reaction is a mixed function oxidase and the first step is the reduction of Cu^{++} by ascorbic acid. Fumarate is also required, possibly to speed the reoxidation of the Cu^{++}. Catalase is necessary to destroy peroxides formed by the auto-oxidation of ascorbate and dopamine. DBH lacks specificity and can *beta*-hydroxylate a wide variety of phenylethylamine derivatives including tyramine (26), phenylethylamine and amphetamine. In addition to noradrenaline, DBH also catalyzes the synthesis of octopamine. This amine is a normally occurring compound present in nerves (27) and is released with noradrenaline as a cotransmitter (Molinoff and Kopin, unpublished data).

DBH is highly localized in the chromaffin granule of the adrenal medulla (22); about half of the enzyme is present in the bound form and half in a soluble form (3). It has also been shown to be present in cell bodies and nerve terminals (28), and is highly localized in the catecholamine-containing granules in heart (39) and splenic nerves (45) and synaptosomes of the brain (5). In the brain the enzyme has the highest localization in brainstem and lowest activity in striatum (table 1). DBH is also present in serum of a variety of mammalian species (55). The enzyme from adrenal gland or tissues shows two electrophoretically distinguished peaks of activity (41). One of these peaks is eliminated with highspeed centrifugation before electrophoresis and presumably represents the bound enzyme. The main peaks of DBH activity from several species have different electrophoretic mobilities, but within species the major peak of activity from all tissues including serum has the same mobility.

Because DBH is a copper-containing enzyme a number of inhibitors that bind this metal have been found. Disulfiram is a potent inhibitor of DBH *in vitro* and the administration of disulfiram causes a rapid and marked fall in the endogenous content of noradrenaline in brain and heart (16). These findings indicate that

TABLE 1

DBH activity in rat brain at birth and in adults

Region	Adult	Newborn
	units/g	
Brainstem	149 ± 5	48 ± 2
Hypothalamus	117 ± 3	20 ± 1.5
Midbrain		
Cortex	54 ± 1	3 ± 1.0
Cerebellum	41 ± 4	14 ± 1.5
Striatum	15 ± 25	

One unit of DBH activity equals 1 nmole product formed per hour (5).

DBH plays an important role in the regulation of noradrenaline synthesis. Tropolone derivatives (16), aromatic and alkyl thioureas (46) and fusaric acid (36) are potent inhibitors of DBH.

Until recently it was difficult to measure DBH in tissue because the existing methods lacked sensitivity and endogenous inhibitors interfered with the precise measurement of the enzyme. A sensitive and convenient procedure has been developed that made it possible to measure DBH where it has never been reported previously (29). The assay uses either phenylethylamine or tyramine as a substrate and is based on the sequential conversion of the *beta*-hydroxylated product formed by DBH to a radioactively labeled N-methylated derivative with phenylethanolamine-N-methyl transferase (PNMT) and ^{14}C-methyl-S-adenosylmethionine as follows: phenylethylamine $\xrightarrow{\text{DBH}}$ phenylethanolamine + ^{14}C-methyl-S-adenosylmethionine $\xrightarrow{\text{PNMT}}$ ^{14}C-methyl-N-phenylethanolamine. The N-methylated derivatives are separated by solvent extraction and their radioactivity is determined. Endogenous inhibitors of DBH are inactivated by the addition of cupric ions.

With this assay DBH was found to be highly localized in cell bodies of sympathetic neurons and nerve terminals (28). It had an unequal distribution in brain (5, 40). It has highest concentrations in brainstem and hypothalamus and low levels in the striatum (table 1). DBH is distributed in brain neurons with a regional pattern of activity that parallels levels of noradrenaline (40). This is reflected by the almost complete disappearance of DBH after the intracisternal administration of 6-hydroxydopamine (40). DBH activity in the cortex is dependent on the integrity of the structure in the lateral hypothalamus.

A study of the development of DBH in the rat brain was examined (5). DBH was detected in the brain of the rat fetus at 15 days gestation and a gradual increase in enzyme activity occurs with maturation. The appearance of DBH precedes the presence of noradrenaline by several days. The enzyme first appears in the caudal brain areas where the cell bodies are localized. With maturation there is a progressive increase in DBH activity and a shift of distribution to the rostal parts of the brain where the nerve terminals are localized (table 1).

Neural Induction of Dopamine-β-hydroxylase (DBH)

Previous studies on the regulation of the biosynthetic enzymes of catecholamines have shown that the administration of drugs which lower blood pressure such as reserpine, phenoxybenzamine and 6-hydroxydopamine cause an increased activity of tyrosine hydroxylase and PNMT in the adrenal gland (31, 32). Increase in tyrosine hydroxylase activity also occurred after prolonged psychosocial stimulation (1), repeated immobilization stress, and cold (23, 48). The administration of reserpine to rabbits caused an initial fall in DBH activity followed by a rise to above normal values in the adrenal (52). The increase in enzyme activity after drug-induced splanchnic nerve activity was shown physiologically by the increased formation of ^{14}C-catecholamine from ^{14}C-tyrosine after the administration of 6-hydroxydopamine (30). When presynaptic nerves innervating the adrenal gland or sympathetic ganglia were cut, increases in enzyme activity were abolished (49, 50). Ganglionic blocking agents also prevented the increase in tyrosine hydroxylase (35). Acetylcholine given together with eserine caused an elevation in tyrosine hydroxylase activity in the adrenal (37). Increased tyrosine hydroxylase activity resulting from reserpine is blocked by inhibiting protein synthesis (33). These experiments indicate that tyrosine hydroxylase is induced by a transsynaptic mechanism.

The availability of a sensitive assay for DBH made it possible to study changes in DBH activity caused by reserpine in rat heart, salivary gland, sympathetic ganglia and adrenal gland. The administration of reserpine for 6 days caused an increase in DBH in the sympathetic ganglia (stellate) and nerve terminals in the heart (28) and adrenal gland (3) (fig. 1), but not in brain. When the preganglionic nerve to the superior ganglia was cut unilaterally followed by the administration of reserpine there was an elevation in DBH in the innervated but not in the decentralized ganglia (fig. 1). Treatment with ganglionic blocking agents also prevented the rise of DBH in sympathetic ganglia (Molinoff, Brimijoin and Axelrod, unpublished data). The administration of cycloheximide, a protein synthesis inhibitor, blocked the reserpine-induced increase in DBH in the stellate ganglia. These experiments indicated that DBH activity is induced in nerves by a transsynaptic mechanism involving new protein synthesis. Additional evidence that reserpine causes an increase in new DBH molecules is the observation that this drug causes an increased rate of incorporation of ^{3}H-leucine into DBH measured by immunoabsorption (18). When nerve terminals were destroyed with 6-hydroxydopamine, the increase in ganglia DBH induced by reserpine was blocked (fig. 1); this indicates that nerve terminals influence the biosynthetic activity of the cell body (3). 6-Hydroxydopamine also causes a fall of ganglia DBH as well as an almost complete disappearance of the enzyme in tissue (3).

After inhibition of protein synthesis there is a fall of DBH activity in the ganglia (Molinoff, Brimijoin and Axelrod, unpublished data). This probably reflects the rapid transport of DBH from the cell body to the nerve terminal *via* the axon (25). It is also a measure of the rate of turnover of this enzyme. The time constant in the fall of DBH in ganglia is the same in normal and reserpine-treated rats and is rapid with a 50% fall in about 15 hr.

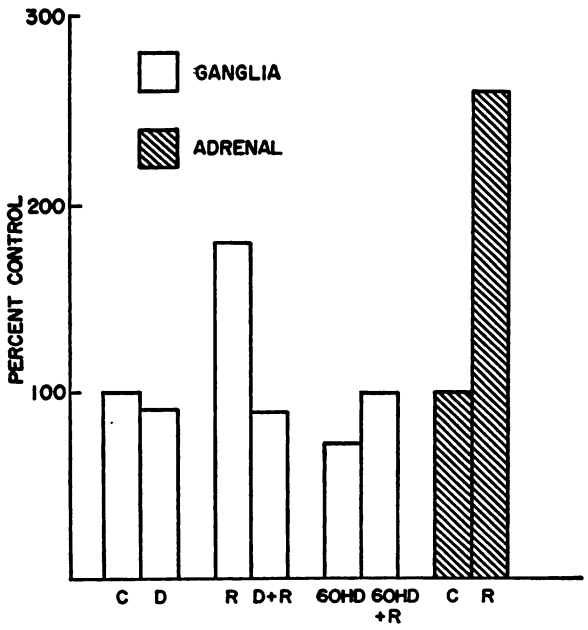

FIG. 1. Transsynaptic induction of DBH. Rats were treated with reserpine, 2.5 mg/kg subcutaneously 48 and 24 hr, and 6-hydroxydopamine 136 mg/kg intravenously 96 and 72 hr before killing the animals. In denervation experiments superior cervical ganglia were decentralized unilaterally 9 days before drug administration. C, control; D, denervated; R, reserpine; and 6OHD, 6-hydroxydopamine. Results are expressed as percent of control values (3, 28).

Rat superior cervical ganglia can be maintained in organ culture for several days (42). Elevation of the potassium concentration in the media causes a marked elevation of DBH in the ganglia. This increase in enzyme activity is blocked by the addition of cycloheximide to the media. These results suggest that high potassium induces an increase in new enzyme protein. Increased potassium causes a depolarization of these neuronal membranes, and an induction of DBH might be a result of catecholamine release. Although acetylcholine and eserine have been shown to increase the activity of DBH in denervated adrenals (37) it does not appear that activation of a cholinergic receptor is essential for the induction of the enzyme in sympathetic nerves since a high potassium concentration can also increase enzyme activity in the absence of neuronal influences. Induction of DBH and tyrosine hydroxylase by reserpine can be inhibited by the administration of dopa or monoamine oxidase inhibitors (Molinoff, Brimijoin and Axelrod, unpublished data). Both of these procedures elevate catecholamine levels in nerve and it is possible that catecholamines can depress the formation of biosynthetic enzymes. A finding consistent with this hypothesis is the observation that when the tissue noradrenaline is decreased by α-methyl-*p*-tyrosine or reserpine there is an increase in DBH (Molinoff, Brimijoin and Axelrod, unpublished data).

Hormonal Control of Dopamine-β-hydroxylase (DBH)

Hypophysectomy has been shown to reduce the activity of PNMT (62) and tyrosine hydroxylase (34) in the adrenal. Adrenocorticotropic hormone (ACTH) can restore both PNMT and tyrosine hydroxylase in hypophysectomized rat adrenals. Dexamethasone, a potent corticoid, increases adrenal PNMT (62) but not tyrosine hydroxylase (34) in hypophysectomized rats.

The effect of removal of the pituitary on adrenal DBH was examined (54). Three weeks after hypophysectomy adrenal DBH was found to be decreased by 70% of the age-matched sham-operated animals (fig. 2) and 50% of the weight-matched controls. The administration of ACTH to hypophysectomized rats resulted in an elevation of adrenal DBH (fig. 2) but dexamethasone had little or no effect on the enzyme. The levels of the enzyme however did not reach normal values even after the repeated administration of ACTH for 10 days. The effects of hypophysectomy on DBH could not be due to changes in adrenal cortex since almost all of the enzyme activity was confined to the adrenal medulla.

When rats were subjected to repeated immobilization stress the levels of adrenal DBH were markedly elevated (14) (fig. 2). The stress-induced elevation of DBH was partially prevented by denervation (fig. 2) or hypophysectomy. All

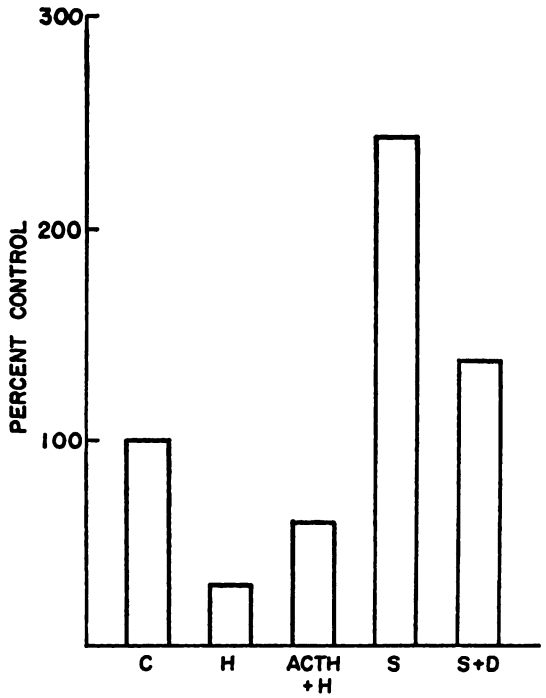

FIG. 2. Hormonal and neural effects of DBH in rat adrenals. Rats were hypophysectomized (H) and after 4 weeks were given four units ACTH daily for 5 days (54). Rat adrenals were denervated (D) unilaterally and subjected to daily immobilization stress (S) for 7 days (14). Results are expressed as percent of control (C) DBH values.

of these observations indicate that DBH in the adrenal is under both neuronal and hormonal control.

Release of Dopamine-β-hydroxylase (DBH)

Stimulation of adrenal medulla by acetylcholine caused a release of not only catecholamines but also adenosine triphosphate (ATP) (8), chromogranin proteins (2), and DBH (53). DBH is highly localized in the chromaffin granule of the adrenal medulla (25) where it is present in a bound and soluble form (20). On chemical stimulation of the adrenal, DBH is released together with catecholamines in the same proportion as was found in the soluble portion of the lyzed vesicles (53). This indicated that the catecholamines were released from the adrenal gland by exocytosis.

Stimulation of the splenic nerve of cow and cat led to the release of noradrenaline and DBH (12, 13, 44). When the nerves to the spleen were stimulated electrically the ratio of the amine to DBH was many times that found in vesicles isolated from splenic nerves. This raised questions as to whether noradrenaline is released from the nerves by exocytosis. This would involve a fusion of the noradrenaline-containing vesicle with the neuronal membrane followed by a discharge of the soluble portion of its contents to the exterior through an opening of the cell membrane large enough to allow DBH (mol wt 290,000) to pass through. The sensitive assay for DBH and ability to stabilize the enzyme with albumin once it was released enabled us to establish that DBH and noradrenaline are discharged from sympathetic nerve terminals by a process of exocytosis (59). Hypogastric nerves attached to guinea pig vas deferens were stimulated electrically *in vitro* and the amount of DBH and noradrenaline released into the bath fluid was increased. To quantitatively measure the noradrenaline released it was necessary to block neuronal uptake by phenoxybenzamine or desmethylimipramine. The amount of noradrenaline and DBH released into the bath fluid was proportional (59). The ratio of the noradrenaline to DBH in the bath fluid was found to be the same as that of the amine to the soluble DBH in the vas deferens. These observations are compatible with the release of the neurotransmitter and DBH by a process of exocytosis. Absence of calcium prevented the release of DBH from perfused spleen when splenic nerve was stimulated (44). DBH and noradrenaline after nerve stimulation can be enhanced by increasing the normal calcium concentration to 7 mM (21) (fig. 3). The elevated release of DBH was blocked by prostaglandin E_2 (fig. 3). Phenoxybenzamine also increased the release of DBH after nerve stimulation but had no effect on the unstimulated preparation (7, 21) (fig. 3). Low concentrations of prostaglandin (1/6 that of phenoxybenzamine) also caused a decrease in the release of DBH after phenoxybenzamine. The elevated release of the enzyme by phenoxybenzamine only when the nerve is stimulated would suggest that the drug allows the nerve to remain in a conformational state that allows larger molecules to be secreted for a longer period of time. Prostaglandin has been shown to block the noradrenaline release from the perfused cat spleen with increased calcium concentration (19). Prostaglandin

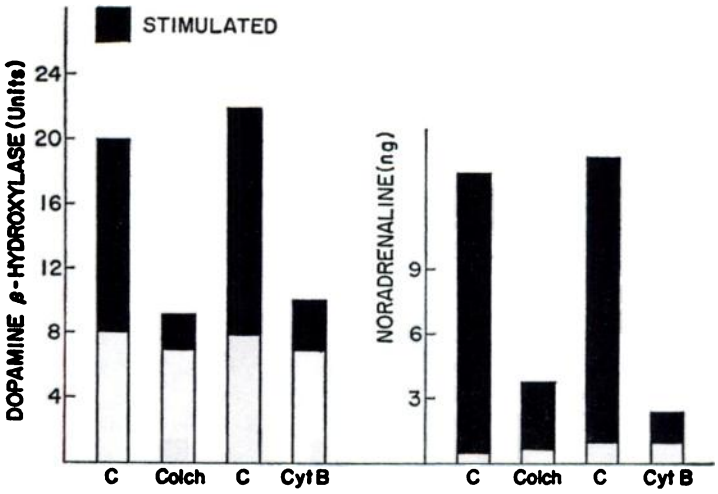

FIG. 3. Release of DBH from sympathetic nerves, effect of Ca^{++}, phenoxybenzamine and prostaglandin. Hypogastric nerves of guinea pig vas deferens were stimulated for 30 min in medium containing various amounts of Ca^{++}, phenoxybenzamine (PB) (3×10^{-6} M) or prostaglandin E_2 (PGE_2) (1.8×10^{-6} M), and medium assayed for DBH. The lower open bars represent bath concentrations of DBH and shaded areas represent DBH released after nerve stimulation (21).

may interfere with the actions of Ca^{++} and thus might influence the calcium-dependent secretion of noradrenaline and DBH.

The release by exocytosis requires the fusion of vesicular and neuronal membrane followed by the formation of a large enough opening to allow DBH to be discharged. Microtubules have been shown to be involved in the liberation of various intracellular stored products such as insulin from the *beta* cells of the pancreas (24), thyroid-stimulating hormone (TSH) induced release of iodine-131 from thyroid gland (61), histamine release from most cells (15) and catecholamine release from the adrenal medulla (38). These findings suggested that microtubules may be implicated in release of DBH from sympathetic nerve terminals. Microtubules can be disaggregated by alkaloids such as colchicine and vinblastin (43). Treatment of vas deferens with colchicine almost completely inhibited the release of DBH and noradrenaline (fig. 4) when the hypogastric nerves were stimulated (47). This alkaloid had no effect on the spontaneous release of the enzyme or noradrenaline, nor did it change the ratio of DBH to noradrenaline released. Vinblastin also blocked the release of DBH. In another experiment the effect of cytocholasin B (a fungal metabolite that disrupts microfilaments) (4) on the release of DBH was examined. This compound also inhibited the release of the neurotransmitter and DBH (fig. 4). These results indicate that both microtubules and microfilaments are necessary components in the exocytosis induced by depolarization. Microtubules have been shown to be necessary for the rapid proximodistal flow of noradrenaline from the cell body to the nerve terminals (6). Microtubular proteins are presumed to function as a cytoskeleton and they

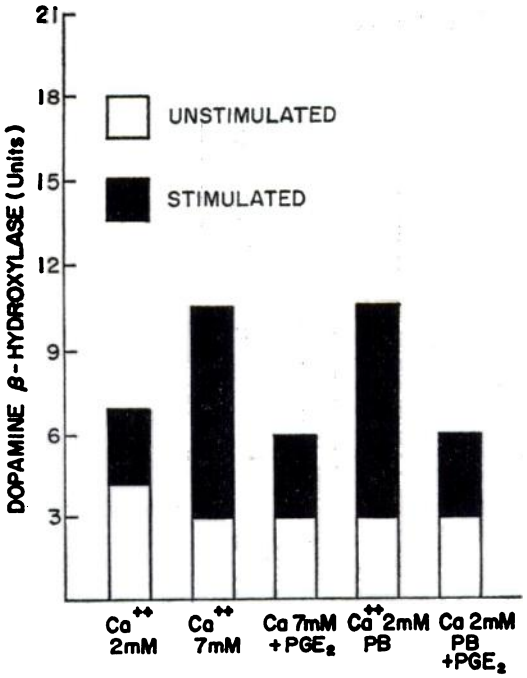

FIG. 4. Inhibition of release of DBH and noradrenaline from sympathetic nerves by colchicine and cytocholasin B. Hypogastric nerve to the guinea pig vas deferens was stimulated for 60 min in the medium containing Ca 7.0 mM and phenoxybenzamine in the absence (C) or presence of colchicine (Colch) (1×10^{-6} M) or cytocholasin B (Cyto B) (3 μg/ml) and medium examined for DBH and noradrenaline. The lower bars represent DBH activity and noradrenaline in incubation medium of unstimulated organs, and dark areas show activities induced by nerve stimulation (47).

might serve to direct the nerve vesicles to the proper sites of the neuronal membrane where release occurs. Cytocholasin B disrupts microfilaments that can serve as contractile elements in cells (60) and also inhibits release by exocytosis. The contractile microfilaments have been proposed to be activated by calcium in non-muscle cells (60). The release of DBH and noradrenaline also requires calcium. It is possible that calcium activates a contraction mechanism in the neuronal membrane to produce an opening which allows the discharge of the neurotransmitter as well as large molecules.

Dopamine-β-hydroxylase in Plasma

The release of DBH from sympathetic nerves and the adrenal medulla suggested that it might overflow into the circulation. The blood was examined for DBH and was found to be present in plasma of man and rat, but not in the cellular elements (11, 55). The plasma DBH and partially purified DBH from adrenal medulla were compared (55). Both had the same requirements for ascorbic acid, fumarate and oxygen. Human adrenal DBH and serum enzyme also had the same electrophoretic mobility. The K_m values for the substrate in

man and rat sera were similar and both were the same as that obtained from rat adrenal gland and stellate ganglia. DBH activity in human plasma was about 50 times that of rat plasma. The serum DBH in man showed a 15-fold spread in enzyme activity (56). Plasma DBH increased with age in normal human population (11, 56). There was about a 10-fold increase in enzyme activity from birth to 10 to 19 years of age, and there was no change in activity in decades up to 40 years of age.

The source of plasma DBH could be the adrenal gland, sympathetic neurons or both. The effect of 6-hydroxydopamine (a compound that destroys most of the sympathetic nerve terminals, but leaves the adrenal medulla unaffected) (51) on rat serum DBH was examined (57). The administration of 6-hydroxydopamine caused a 25% reduction in serum DBH. This decrease in enzyme activity in blood persisted in rats whose adrenal glands were demedullated. Removal of the adrenal gland did not lower serum DBH activity. These experiments suggested that the serum DBH arises from sympathetic nerve terminals and not from the adrenal gland.

When rats were subjected to repeated immobilization stress there was a sharp elevation in serum DBH (58). The stress-induced increase in serum DBH was still present after adrenal demedullation and adrenalectomy. It thus appears that the elevated serum DBH arises mainly from sympathetic nerves. Human beings subjected to various stress, exercise, cold pressor or psychological (Wooten and Cardon, unpublished data) produced a rapid elevation in plasma DBH.

Removal of the pituitary gland also caused an elevation of serum DBH in rats while the administration of DOCA led to a reduction of enzyme activity (Lamprecht and Wooten, unpublished data). In hypophysectomized rats posterior pituitary extract but not ACTH caused a decrease in plasma DBH. It thus appears that procedures that cause high plasma sodium levels elevate serum DBH.

Serum DBH was examined in a number of diseases. The amount of enzyme in the blood was significantly decreased in familial dysautonomia (56). About 25% of patients with this disease had no circulating DBH activity and the mothers of the subjects without serum DBH had markedly lower serum DBH. This suggests a subgroup of DBH negative patients with dysautonomia. No difference in DBH activity was found in depressed subjects or hypertensive patients (Lamprecht and Wooten, unpublished data).

Conclusions

Increased sympathetic nervous activity induced by drugs and stress elevates dopamine-β-hydroxylase activity in cell body, nerve terminal and adrenal gland. The increase in enzyme activity depends on continuing protein synthesis and is a transsynaptic event. Maintenance of dopamine-β-hydroxylase activity in the adrenal medulla is dependent on an intact pituitary gland.

Dopamine-β-hydroxylase and noradrenaline are released by a process of exocytosis. Release of the neurotransmitter and DBH is enhanced by phenoxybenzamine and calcium and blocked by prostaglandins, colchicine and cytocholasin B.

Dopamine-β-hydroxylase is present in serum of man and other mammalian species. Serum DBH arises mainly from sympathetic nerve terminals. It is elevated by stress and hypophysectomy and reduced in familial dysautonomia.

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