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Significance of Plasma Dopamine β-Hydroxylase Activity as an Index of Sympathetic Neuronal Function

(norepinephrine/adrenergic neuronal blockade/bretylium/chlorisondamine/phenoxybenzamine)

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ABSTRACT **Plasma norepinephrine and dopamine β-hydroxylase (EC 1.14.17.1) activity were measured in rats. Adrenergic neuron blockade with bretylium for 4 hr and ganglion blockade with chlorisondamine for 72 hr lowered plasma norepinephrine. Neither treatment altered plasma dopamine β-hydroxylase activity. Phenoxybenzamine for up to 48 hr markedly raised plasma norepinephrine and transiently lowered plasma dopamine β-hydroxylase at 24 hr. Prolonged pharmacological modification of sympathetic nervous activity and plasma norepinephrine were not attended by parallel changes in circulating dopamine β-hydroxylase activity. Plasma dopamine β-hydroxylase activity does not appear to be a sensitive index of prolonged alterations in sympathetic neural activity. Norepinephrine in plasma, however, appears to reflect sensitively and accurately the rate of release of the neurotransmitter.**

Stimulation of postganglionic adrenergic nerves results in release by exocytosis of dopamine-β-hydroxylase [3,4-dihydroxyphenylethylamine, ascorbate:oxygen oxidoreductase (β-hydroxylating), EC 1.14.17.1; abbreviated DBH] together with the neurotransmitter norepinephrine (1). Plasma DBH appears to be derived at least in part from sympathetic neurons since treatment with 6-hydroxydopamine (6 OH DA), but not adrenalectomy, lowers levels of the enzyme (2). It has been suggested that the activity of the enzyme in plasma may be an index of sympathetic nervous activity (2, 3). Some workers have reported acute rises in plasma DBH activity following immobilization stress in rats (4) and the cold pressor test in man (5), but others have been unable to demonstrate a close relationship between sympathetic activity and plasma DBH (6). Destruction of peripheral adrenergic endings with 6-hydroxydopamine reduced plasma DBH activity of rats by only 20% (2), and in human hypertensives prolonged treatment with a variety of antiadrenergic drugs lowered blood pressure, but did not influence serum DBH activity (6). Schanberg *et al.* (3), however, found a correlation of urinary excretion of catecholamines and plasma DBH activity in man.

The present study was designed to investigate the relationship between plasma DBH activity and plasma norepinephrine following treatment with drugs that profoundly modify sympathetic neuronal activity.

METHODS

All studies were performed on 180 to 200-g male Sprague–Dawley rats (Zivic Miller Labs, Allison Park, Pa.). Groups of six to ten intact or adrenalectomized rats were treated by intraperitoneal injections with either bretylium (50 mg/kg), phenoxybenzamine (10 mg/kg, three times daily), or chlorisondamine (2 mg/kg, three times daily). Control groups received intraperitoneal 0.9% sodium chloride vehicle. Rats were anesthetized by injection of sodium methohexital (10 mg/kg) into the lateral tail vein and blood was collected by cardiac puncture within 20 sec of anesthesia and added to heparinized tubes. After centrifugation, the heparinized plasma was frozen and stored at −20° until assay.

Plasma DBH activity was measured by the method of Weinshilboum and Axelrod (7) using 50-μl aliquots of plasma and 1.0 mM tyramine as substrate. DBH activity is expressed as units (1 nmole of octopamine formed per milliliter of plasma per hour of incubation).

Proteins were precipitated by the addition of 5 μl of perchloric acid to 0.4 ml of plasma and, after centrifugation, norepinephrine in 100-μl aliquots of the clear supernatant solution was assayed by an enzymatic radiometric method. This assay, which utilizes a purified preparation of phenylethanolamine *N*-methyl transferase (8) and tritiated *S*-adenosylmethionine (New England Nuclear Corp.) is a modification of earlier methods (9, 10). Tritiated epinephrine formed in the reaction is adsorbed onto alumina and eluted with 0.1 N perchloric acid. After addition of unlabeled epinephrine and *S*-adenosylmethionine carrier, and precipitation of the residual labeled adenosylmethionine with 25% phosphotungstic acid, the tritiated product is extracted into 1% diethyl-dihexyl-phosphoric acid in toluene and the tritium is assayed in a liquid scintillation counter. Duplicate samples were assayed, as were additional samples containing 0.1–1 ng of norepinephrine as internal standards. Blanks consisting of 0.1 N perchloric acid were run simultaneously through all stages of the assay and were equivalent to about 0.02 ng of norepinephrine.

RESULTS

The availability of a highly sensitive and specific radiometric method for assay of catecholamines has made possible demonstration of a fall in norepinephrine after administration of drugs that block release of the sympathetic neurotransmitter.

A single dose of bretylium (50 mg/kg, intraperitoneal), which impairs sympathetic nerve transmission and prevents release of neurotransmitters from adrenergic nerves (11), lowered plasma norepinephrine in both intact and adrenalectomized rats (Fig. 1). The more prominent and prolonged fall in plasma catecholamine levels of adrenalectomized animals may reflect the absence of compensatory amine release from

Abbreviation: DBH, dopamine β-hydroxylase.

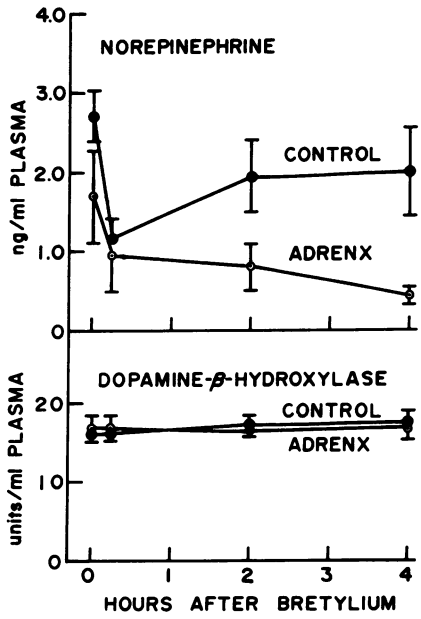

FIG. 1. Plasma norepinephrine and DBH activity (mean ± SEM) after bretylium 50 mg/kg intraperitoneally in groups of intact and adrenalectomized (adrenx) rats ($n = 6$).

the adrenal medulla. Although plasma norepinephrine was lowered, plasma DBH activity did not change significantly in intact or adrenalectomized animals (Fig. 1).

To investigate the effect of more prolonged sympathetic blockade, we administered chlorisondamine, a long-acting ganglionic blocking agent (12), three times daily, at 8-hr intervals, for up to 72 hr. Rats were killed 8 hr after the last dose of the ganglionic blocker. Plasma norepinephrine was markedly reduced throughout this interval, but there was no change in plasma DBH activity (Fig. 2).

In another group of rats, the actions of the alpha adrenoceptor blocker phenoxybenzamine (10 mg/kg intraperitoneally three times daily) were investigated. Phenoxybenzamine in isolated tissues enhances release of norepinephrine and dopamine β-hydroxylase from nerve endings (1). Plasma norepinephrine increased from control levels of 2.30 ± 0.24 ng/ml to 8.80 ± 2.16 ng/ml at 8 hr and significantly increased levels were maintained for up to 48 hr ($P < 0.01$) (Fig. 2). Plasma DBH activity was unchanged 8 hr after phenoxybenzamine; after 24 hr, DBH activity was slightly, but significantly, lower ($P < 0.05$) than in controls, but DBH activity in plasma was again normal at 48 hr.

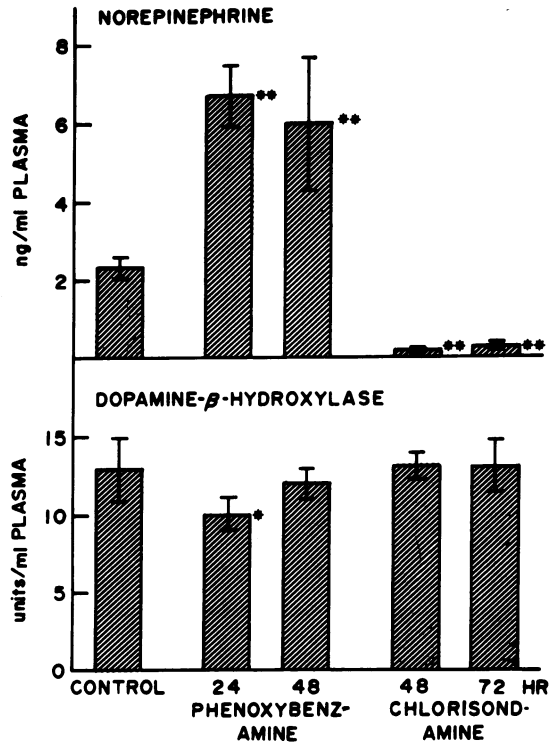

FIG. 2. Plasma norepinephrine and DBH activity (mean ± SEM) in control rats and after 48 or 72 hr treatment with chlorisondamine (2 mg/kg, intraperitoneally three times daily) or after 24 or 48 hr phenoxybenzamine (10 mg/kg, intraperitoneally three times daily). $*P < 0.05$, $**P < 0.01$ when treated groups are compared with controls with an unpaired t test.

DISCUSSION

Agents that raise or lower plasma norepinephrine and that have been demonstrated *in vitro* to alter DBH release in a parallel fashion, *in vivo* do not change plasma DBH activity. The only significant change in plasma DBH in the present study was a fall after phenoxybenzamine associated with marked increases in plasma norepinephrine.

Rush and Geffen (13) using ^{125}I-labeled purified bovine DBH, reported a plasma half-life of 3–4 hr when the enzyme was given intravenously to lambs. Even if the half-life were 24 hr, blockade of DBH release would be expected to lower plasma DBH within a relatively short time, and certainly within 1 day. Prolonged blockade of release of norepinephrine from sympathetic nerves, however, is not associated with diminished plasma DBH activity. This suggests that stimulation-coupled release of DBH from sympathetic nerves is not essential for discharge of the enzyme from the nerve ending into the plasma. The enzyme might be derived from other sources, but there is no evidence that DBH is present in any nonadrenergic tissue.

DBH is synthesized in the cell bodies of postganglionic sympathetic neurons, is incorporated into vesicles, and passes by rapid axonal transport to the nerve endings (14). Over any prolonged period the amount of DBH available for release into the plasma is limited by these processes. Axonal flow is not dependent on preganglionic neuronal connections or the passage of nerve impulses (15, 16). In the isolated vas deferens preparation there is considerable spontaneous release of DBH which is not influenced by blockade of axonal transport with colchicine (17). Most of the DBH at sympathetic nerve endings is not released by exocytosis since it is bound to the vesicular membrane. The fate of this DBH is not known. Our results suggest that, in addition to exocytosis, considerable quantities of DBH may be released from nerve endings by a process not dependent on nerve stimulation. Chlorisondamine does not alter DBH activity in the rat stellate ganglion (18). If DBH synthesis, axonal transport, and incomplete intraneuronal destruction continue unchanged, then spontaneous (nonimpulse-coupled) release could account for the maintenance of levels of plasma DBH. When DBH is released by exocytosis during a sudden increase in sympathetic neuronal activity, then plasma DBH levels may increase transiently. This results in depletion of a portion of the DBH at the

nerve endings. The slight fall in plasma DBH after 24 hr of phenoxybenzamine administration may be due to such depletion of the releasable enzyme from nerve endings with delay in its replenishment by newly synthesized DBH from the cell bodies.

The observations on the lack of correlation of plasma norepinephrine and DBH levels in the rat suggest that DBH may be of limited value in assessing sympathetic neuronal activity. This has not yet been assessed in other species, but there are indications that this may be true in man (6).

Until further information is available on the factors controlling synthesis, release, and degradation of DBH, it appear advisable to exercise caution in using measurements of plasma DBH activity as an index of prolonged changes in sympathetic neuronal activity. The plasma level of norepinephrine appears to be a more accurate and responsive index of sympathetic activity.

J.L.R. is a Medical Research Council of Great Britain Wellcome Travelling Fellow in clinical pharmacology.

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