

Neuronal Source of Plasma Dopamine

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BACKGROUND: Determinants of plasma norepinephrine (NE) and epinephrine concentrations are well known; those of the third endogenous catecholamine, dopamine (DA), remain poorly understood. We tested in humans whether DA enters the plasma after corelease with NE during exocytosis from sympathetic noradrenergic nerves.

METHODS: We reviewed plasma catecholamine data from patients referred for autonomic testing and control subjects under the following experimental conditions: during supine rest and in response to orthostasis; intravenous yohimbine (YOH), isoproterenol (ISO), or glucagon (GLU), which augment exocytotic release of NE from sympathetic nerves; intravenous trimethaphan (TRI) or pentolinium (PEN), which decrease exocytotic NE release; or intravenous tyramine (TYR), which releases NE by nonexocytotic means. We included groups of patients with pure autonomic failure (PAF), bilateral thoracic sympathectomies (SNS-x), or multiple system atrophy (MSA), since PAF and SNS-x are associated with noradrenergic denervation and MSA is not.

RESULTS: Orthostasis, YOH, ISO, and TYR increased and TRI/PEN decreased plasma DA concentrations. Individual values for changes in plasma DA concentrations correlated positively with changes in NE in response to orthostasis ($r = 0.72, P < 0.0001$), YOH ($r = 0.75, P < 0.0001$), ISO ($r = 0.71, P < 0.0001$), GLU ($r = 0.47, P = 0.01$), and TYR ($r = 0.67, P < 0.0001$). PAF and SNS-x patients had low plasma DA concentrations. We estimated that DA constitutes 2%–4% of the catecholamine released by exocytosis from sympathetic nerves and that 50%–90% of plasma DA has a sympathoneuronal source.

CONCLUSIONS: Plasma DA is derived substantially from sympathetic noradrenergic nerves.

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Human plasma contains 3 endogenous catecholamines—dopamine (DA),² norepinephrine (NE), and epinephrine (EPI). NE in the bloodstream is derived mainly from networks of sympathetic nerves enmeshing blood vessels throughout the body and pervading organs such as the heart and kidneys. Plasma NE concentrations therefore have been used widely to indicate sympathetic nervous system activity. Plasma EPI concentrations generally reflect neural outflow to the adrenal medulla and consequent secretion from adrenomedullary chromaffin cells into the bloodstream.

Whereas the sources of plasma NE and EPI are well known, those of the third endogenous catecholamine, DA, remain poorly understood. Plasma DA concentrations normally are very low—about 0.1 nmol/L—and plasma DA concentrations are rarely reported.

One potential source of plasma DA is the diet (pathway 1 in Fig. 1). Plasma DA increases after ingestion of a standard meal and decreases after prolonged fasting (1). Ingestion of a standard meal increases plasma DA sulfate concentrations by more than 50-fold (1). The liver removes and metabolizes virtually all the catecholamine delivered to it by the portal vein. Therefore, although the gastrointestinal tract constitutes the main site of DA production in the body (2), very little of the free DA in the systemic circulation is derived from the gut.

Another potential source of plasma DA is nonneuronal uptake and decarboxylation of circulating dihydroxyphenylalanine (DOPA), catalyzed by L-aromatic-amino-acid decarboxylase (LAAAD, pathway 2 in Fig. 1). Such a process is known to be prominent in the kidneys (3); however, since there is no significant arterial-renal venous increment in plasma DA in humans (2), the renal contribution to plasma DA is probably very small. Considering the extremely high plasma concentration of DA sulfate compared with that of free DA, plasma DA might reflect a slight amount of deconjugation of DA sulfate, to regenerate the free catecholamine in nonneuronal cells.

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² Nonstandard abbreviations: DA, dopamine; NE, norepinephrine; EPI, epinephrine; DOPA, dihydroxyphenylalanine; DBH, dopamine- β -hydroxylase; YOH, yohimbine; ISO, isoproterenol; GLU, glucagon; TRI, trimethaphan; PEN, pentolinium; TYR, tyramine; PAF, pure autonomic failure; MSA, multiple system atrophy; POTS, postural tachycardia syndrome; PD, Parkinson disease; SNS-x, thoracic sympathectomy; NCS, neurocardiogenic syncope; DHPG, dihydroxyphenylglycol.

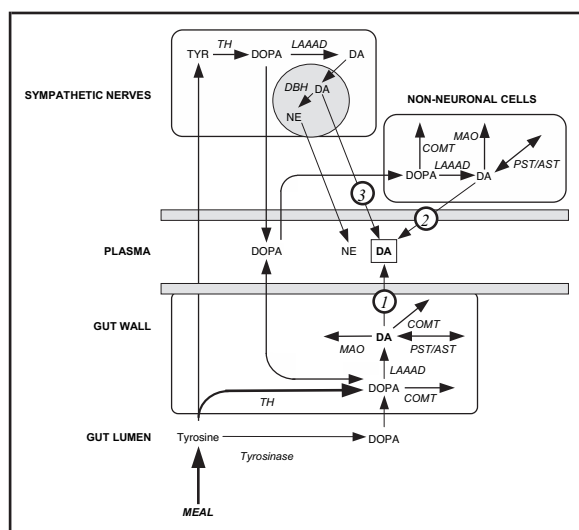


Fig. 1. Potential determinants of plasma DA.

(1), Dietary tyrosine (TY), with conversion to DOPA in the gut wall catalyzed by tyrosine hydroxylase (TH) and then conversion of DOPA to DA by L-aromatic-amino-acid decarboxylase (LAAAD). (2), Nonneuronal uptake of DOPA. (3), TYR hydroxylation via TH in sympathetic noradrenergic nerves. DA can be metabolized by multiple enzymes, including monoamine oxidase (MAO), catechol-O-methyltransferase (COMT), and phenolsulfotransferase (PST). DA sulfate can be converted back to DA via arylsulfatase (AST). In vesicles of sympathetic nerves, DA is converted to NE via DBH, so exocytosis can lead to corelease of NE and DA into the extracellular fluid.

This study focused on a possible third source of free (unconjugated) DA in plasma—sympathetic noradrenergic nerves. According to this concept, DA is coreleased with NE during exocytosis (pathway 3 in Fig. 1). Dopamine- β -hydroxylase (DBH), the enzyme that links DA to NE, is localized in and released from the vesicles in sympathetic nerves. Sympathetic nerves are thought to contain at least 2 vesicular pools—one a storage pool characterized by slow net loss of the vesicular contents by leakage into the axoplasm, and another a release pool with relatively rapid loss by exocytosis and exchange of the vesicular contents with the extracellular fluid (4). The rapid release pool contains newly synthesized NE (5). DA that has not yet been converted to NE by intravesicular DBH might be coreleased with NE during exocytosis.

If plasma DA were derived from sympathetic nerves, one would predict positive correlations between changes in plasma DA and plasma NE concentrations across individual subjects in response to stimuli that alter rates of exocytosis. In this study, we used orthostasis, intravenous infusion of yohimbine (YOH),

infusion of isoproterenol (ISO), and injection of glucagon (GLU) to increase exocytosis. Orthostasis stimulates sympathetic outflow reflexively, resulting in rapid increases in directly recorded sympathetic nerve traffic and correlated increases in plasma NE concentrations (6). YOH, an α -2 adrenoceptor blocker, increases sympathetic outflow and augments NE release for a given amount of sympathetic nerve traffic (7, 8). ISO, a non-selective β -adrenoceptor agonist, increases NE release by occupying stimulatory β -2 adrenoceptors on sympathetic outflows reflexively in response to systemic vasodilation. GLU increases plasma epinephrine concentrations substantially and NE concentrations slightly (10). Ganglion blockers such as trimethaphan (TRI) and pentolinium (PEN) decrease exocytosis, by inhibiting postganglionic sympathetic nerve traffic.

We culled data from subjects in whom the rate of entry of NE into the venous drainage of the heart was measured during right heart catheterization at baseline and during YOH infusion.

We also included infusion of the sympathomimetic amine, tyramine (TYR) (11, 12). NE release evoked by TYR is not calcium dependent and therefore is thought to be by nonexocytotic means. Because contamination of TYR infusates with DA can increase plasma DA levels artifactually (13, 14), we included testing of whether TYR-evoked increments in plasma DA concentrations relate to DA concentrations in the infusate.

We chose patient groups with different, well-characterized autonomic disorders, to provide a range of plasma NE concentrations with which to compare plasma DA concentrations. Pure autonomic failure (PAF) is characterized by diffuse loss of sympathetic noradrenergic nerves, whereas in multiple system atrophy (MSA), sympathetic innervation is generally intact. The 2 diseases differ in plasma NE and its metabolites (15). If plasma DA emanated from sympathetic noradrenergic nerves, then PAF patients would have relatively low plasma DA concentrations.

Bilateral thoracic sympathectomies decrease sympathetic outflows to the head and arms. Noradrenergic innervation of the heart also is decreased (16). We included data from patients who had undergone this procedure.

Postural tachycardia syndrome (POTS) features symptoms and signs of catecholamine excess, despite normal sympathetic nerve traffic during supine rest. The patients have exaggerated increments in sympathetic activity during orthostasis (17) or vasodilator-induced hypotension (18). If plasma DA were derived partly from exocytosis from sympathetic noradrenergic nerves, then the recently reported finding of increased NE and DA concentrations in POTS (19) could

be explained by increased exocytosis for a given amount of sympathetic nerve traffic, decreased neuronal reuptake of catecholamines, or both abnormalities.

Materials and Methods

We reviewed plasma catecholamine data from subjects who had given written informed consent to participate in protocols approved by the Intramural Research Board of the National Institute of Neurological Disorders and Stroke and carried out in the NIH Clinical Center.

Included were 30 patients with Parkinson disease (PD) (18 of whom had neurogenic orthostatic hypotension), 37 with MSA, and 15 with PAF, diagnosed according to generally accepted clinical criteria (20); 5 with bilateral thoracic sympathectomies (SNS-x); 61 with POTS; 14 with neurocardiogenic syncope (NCS); 9 with miscellaneous disorders who did not have neurogenic orthostatic hypotension or evidence of progressive central neurodegeneration; and 41 healthy volunteers. Results about other catechols in these groups have been published (21–24). Comprehensive autonomic function testing differentiated POTS from PAF and PD in all subjects.

Patients with NCS or miscellaneous disorders and healthy volunteers constituted a control group. NCS is associated with normal sympathetic nerve traffic and normal plasma NE concentrations during supine rest (22, 25).

Plasma catecholamines were assayed in our laboratory using liquid chromatography with electrochemical detection after batch alumina extraction (26). The limit of detection for plasma DA was about 2 pg/mL (13 pmol/L) and for NE about 1 pg/mL (6 pmol/L). DA concentrations below the detection limit were assigned a value of zero.

We reviewed arterial plasma DA data from 92 subjects who were off levodopa—10 PD, 32 MSA, 13 PAF, and 37 control. Blood was sampled via a brachial catheter after the subject had been at rest for at least 15 min.

In 206 subjects (26 PD, 37 MSA, 18 PAF, 61 POTS, 64 control), blood was sampled through an arm venous catheter at baseline and after the subject was tilted upright at 90 degrees from horizontal for 5 min. If blood pressure fell rapidly and this was deemed clinically significant, the duration of tilting was <5 min.

In 49 subjects (10 PD, 17 MSA, 7 PAF, 15 control), an i.v. bolus of YOH was administered (0.065 mg/kg/min over 3 min) followed by an infusion at 0.5 μ g/kg/min for 12 min. Blood was sampled through an arm catheter at baseline and at the end of the YOH infusion.

In 51 subjects, the arterial to coronary sinus increment in plasma DA concentrations and cardiac norepi-

nephrine spillover were measured during right heart catheterization at baseline and during YOH infusion.

In 34 subjects (8 PD, 13 MSA, 1 PAF, 12 control), ISO was infused intravenously at increasing doses (3.5, 7.0, 14.0, and 35 ng/kg/min) until a stable heart rate increase of about 25 bpm was attained. Blood was sampled through an indwelling arm catheter at baseline and during ISO infusion at the criterion dose.

In 33 subjects (4 PD, 11 MSA, 5 PAF, 13 control), GLU was injected at a bolus intravenous dose of 1 mg. Blood was sampled through an indwelling arm catheter at baseline and at 1, 2, 3, and 5 min after GLU injection. Catecholamine data after GLU injection were averaged, and responses to GLU were quantified from the baseline value subtracted from the mean value after GLU injection.

In 37 subjects (5 PD, 15 MSA, 6 PAF, 11 control), blood was sampled through an indwelling arm catheter at baseline and during intravenous infusion of TYR at 60 cc/h (1.0 mg/min) for 10 min.

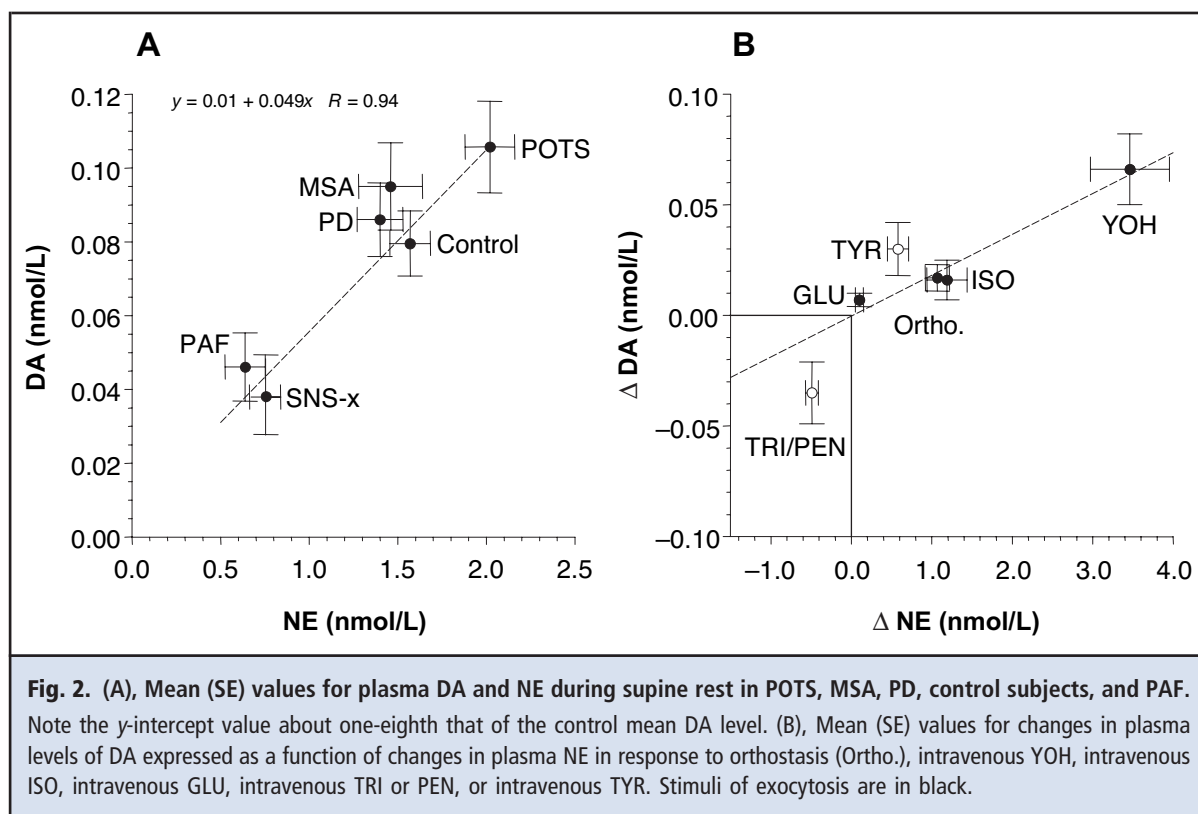
In 27 subjects (6 PD, 7 MSA, 3 PAF, 11 control), blood was sampled through an indwelling arm catheter at baseline and during intravenous infusion of trimethaphan (TRI, 1.0 mg/min) for 10 min or at 15 min after intravenous bolus injection of pentolinium (PEN, 2.5 mg).

We assessed responses of plasma catecholamine concentrations using paired *t* tests and compared patient groups using independent-means *t* tests or analyses of variance, with the Fisher protected least significant difference post-hoc test. We assessed relationships between increments in plasma DA and NE concentrations across individual subjects using linear regression and Pearson correlation coefficients. Data were expressed as mean (SE). A *P* value <0.05 defined statistical significance.

Results

DA was detectable in antecubital venous plasma in 97% of patients with PD, 95% with MSA, 67% with PAF, 100% with SNS-x, 95% with POTS, 86% with NCS, 100% with miscellaneous conditions, and 93% of normal volunteers.

Across all subjects, plasma concentrations of DA correlated positively with those of NE during supine rest, in both arterial and arm venous ($r = 0.24$, $P = 0.009$; $r = 0.40$, $P < 0.0001$) plasma. Mean values for arm venous DA also correlated positively with those of NE across subject groups ($r = 0.94$, $P < 0.01$) (Fig. 2A), with relatively low mean values for DA and NE in PAF and SNS-x and increased mean values in POTS. The PD, MSA, and control groups did not differ in absolute values for plasma DA or NE during supine rest. In sub-



jects undergoing right heart catheterization, the arterial-coronary sinus increment in plasma DA correlated positively with cardiac NE spillover ($r = 0.54$, $P < 0.001$). As indicated in Fig. 2A, the *y*-intercept for the linear regression line of best fit (0.01 nmol/L) corresponded to about one-eighth that of the mean plasma DA concentration in the control group. Among control subjects, plasma DA during supine rest was unrelated to plasma NE ($r = 0.11$).

Plasma concentrations of DA increased significantly in response to orthostasis ($t = 3.2$, $P = 0.002$), YOH infusion ($t = 2.2$, $P = 0.04$), ISO infusion ($t = 2.5$, $P = 0.017$), and TYR injection ($t = 2.9$, $P = 0.007$) but not GLU injection ($P = 0.35$) (Fig. 3). In response to ganglion blockade with TRI or PEN, plasma DA decreased ($t = 2.5$, $P = 0.018$).

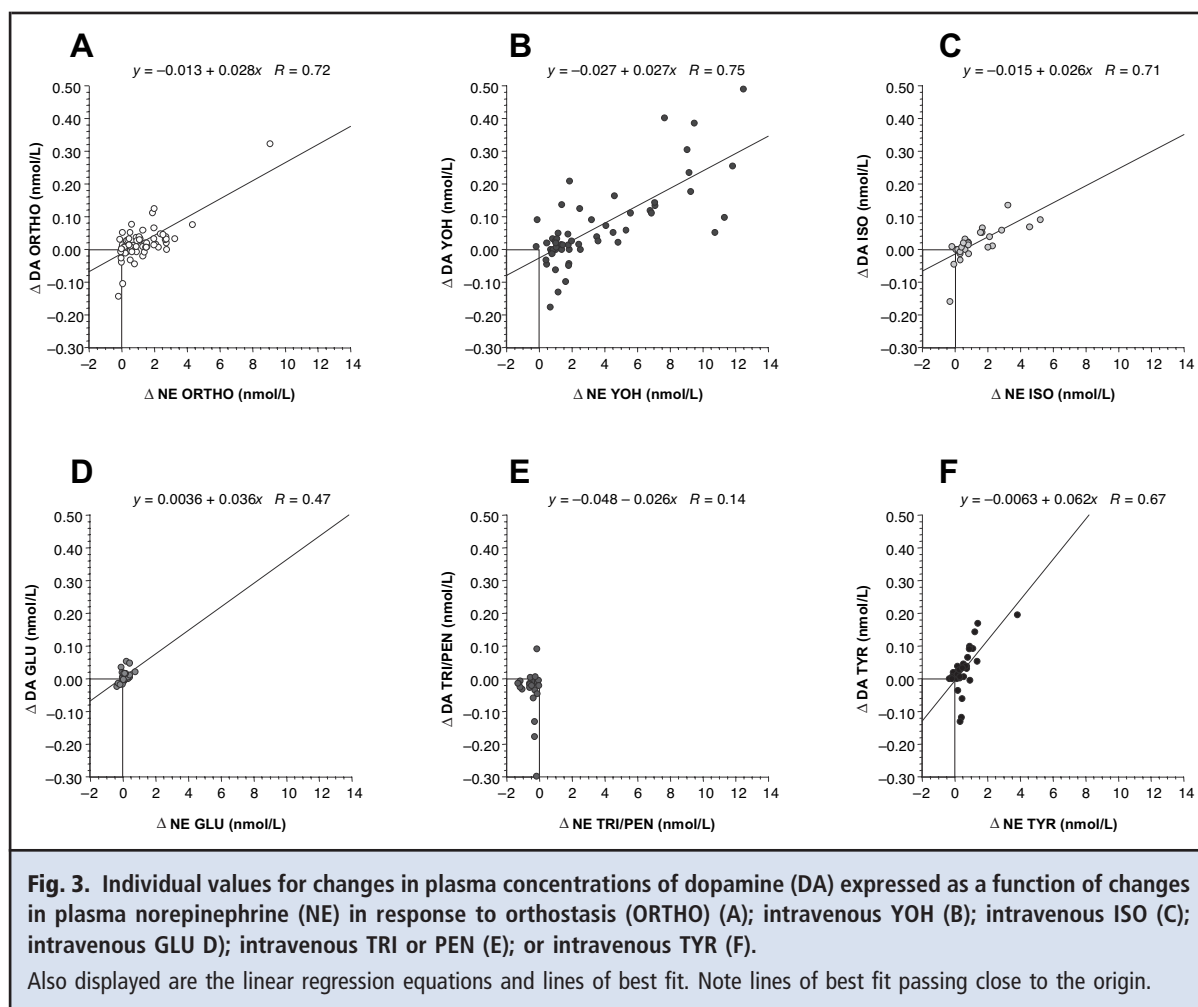
Individual values for changes in plasma DA concentrations were positively correlated with those in NE in response to orthostasis ($r = 0.75$, $P < 0.0001$), YOH ($r = 0.75$, $P < 0.0001$), ISO ($r = 0.72$, $P < 0.0001$), GLU ($r = 0.47$, $P = 0.01$), and TYR ($r = 0.67$, $P < 0.0001$) (Fig. 3). For all stimuli, the *y*-intercept value for the relationship between the increment in plasma DA and that in plasma NE was close to the origin.

During YOH infusion in subjects undergoing right heart catheterization, arterial-coronary sinus differences in plasma DA correlated positively with cardiac

NE spillovers ($r = 0.66$, $P < 0.001$), and the increments in the arterial-coronary sinus difference from baseline correlated positively with the increments in cardiac NE spillover ($r = 0.49$, $P < 0.001$).

In response to TRI/PEN, the extent of decrease in plasma DA seemed somewhat larger than expected for the decrease in plasma NE, and in response to TYR, the extent of increase in DA seemed larger than expected for the increase in NE, compared to responses to other stimuli (orthostasis, YOH, ISO, and GLU) (Fig. 2B). The slope of the line of best fit for the relationship between plasma DA and plasma NE responses to TYR seemed larger than those for responses to orthostasis, YOH, ISO, or GLU (Fig. 3).

During TYR infusion, the increment in plasma DA varied with the DA concentration in the TYR infusate ($r = 0.53$, $P = 0.001$). When data were excluded from subjects who received TYR that contained more than 120 nmol/L of DA, there was no longer a relationship between the increment in plasma DA and the DA concentration in the TYR infusate ($r = -0.14$, $P = 0.49$), but the increment in plasma DA remained positively correlated with that in plasma NE ($r = 0.67$, $P < 0.0001$), and the slope of the line of best fit remained substantially greater than the slopes for orthostasis, YOH, GLU, and ISO.



Discussion

The results of this study support the view that in humans, free (unconjugated) DA in plasma is derived substantially from sympathetic noradrenergic nerves. Patient groups with PAF or SNS-x, conditions that are characterized by loss of sympathetic noradrenergic nerves, had low plasma concentrations of both DA and NE. Conversely, patients with POTS, which is associated with augmented NE release from sympathetic nerves into the plasma (22), had increased plasma concentrations of both DA and NE. A variety of stimuli that release NE, by either exocytotic or nonexocytotic means, from sympathetic nerves increased plasma DA and NE concentrations, and ganglion blockade, which temporarily eliminates sympathetic nerve traffic, decreased plasma DA and NE concentrations. Moreover, in response to all 4 stimuli of exocytosis from sympathetic noradrenergic nerves—orthostasis, YOH, ISO, and GLU—individual values for increments of plasma

DA concentrations were strongly positively correlated with those of plasma NE. Finally, during right heart catheterization, the arterial-coronary sinus difference in plasma DA was correlated positively with cardiac NE spillover, during both supine rest and YOH infusion.

For all stimuli of NE release, the y -intercept value for the relationship between the increment in plasma DA and that in plasma NE was close to the origin. This finding was consistent with a shared source of increments in plasma concentrations of the 2 catecholamines, because if there were other determinants of plasma DA (e.g., production and release of DA but not of NE from DOPA decarboxylation in nonneuronal cells), then the y -intercept value for line of best fit would be above the origin. In healthy humans, infused DA does not affect plasma NE concentrations until a supraphysiologic DA concentration is reached that is far above the concentrations in any of the patient groups or manipulations in the present study (27, 28). Endogenous DA also does not seem to play a modulatory role in NE release (29).

NE infusion, if anything, decreases plasma DA concentrations (30). The most likely explanation for relationships between plasma DA and plasma NE concentrations is therefore a shared source of both catecholamines, such as corelease from sympathetic nerves.

The small slopes for stimuli of exocytosis (0.026–0.036 nmol DA/nmol NE) suggest that DA constitutes only a very small proportion—about 2% to 4%—of the catecholamine released by these stimuli. The results support the inference that the vesicles undergoing exocytosis from sympathetic nerves contain about 25–50 times as much NE as DA.

Across different forms of dysautonomia, chosen to provide a spectrum of NE release from sympathetic nerves, mean plasma DA concentrations varied linearly with mean plasma NE concentrations. From the findings that the PAF and SNS-x groups had mean plasma DA concentrations that were about one-half the mean value in the control group (Fig. 2A), at least half of plasma DA is derived normally from sympathetic noradrenergic nerves. Assuming linear extrapolation to the γ -intercept (0.010 nmol/L in Fig. 2A), and given a normal plasma DA concentration of 0.080 nmol/L, almost 90% of plasma DA would be derived from sympathetic noradrenergic nerves. Most of plasma DA therefore seems to have a sympathoneural source.

The increment in plasma DA during TYR infusion was related to the DA content of the infusate, as noted previously (13). When we excluded data from subjects who received TYR infusates containing more than 120 mmol/L DA, there was no relationship between the increment in plasma DA during TYR infusion and the DA concentration in the infusate, but there was still a larger plasma DA response than expected for the NE response.

The relatively large plasma DA responses for plasma NE responses to TYR (Figs. 2 and 3A), might be explained by TYR not only displacing DA with NE from vesicles but also competing with cytoplasmic DA for monoamine oxidase and for the vesicular monoamine transporter, enhancing exit of DA.

One way to distinguish a reverse NET transport effect from an exocytosis effect would be to track extracellular fluid concentrations of NE and of its neuronal metabolite, dihydroxyphenylglycol (DHPG). In humans, increases in exocytosis result in about the same absolute increases in plasma NE and DHPG, because the latter reflects reuptake of the released NE (31). In contrast, displacement of NE from vesicles into the axoplasm generates DHPG independently of NE release into the extracellular fluid. NE could build up sufficiently in the axoplasm to exit the nerve by reverse NET transport, whereas DHPG, a glycol, diffuses readily across membranes. Augmented net leakage of NE from vesicles into the axoplasm therefore would be

expected to result in larger absolute increments in plasma DHPG than NE levels. Such a pattern occurs with TYR infusion (32).

The concept of calcium-independence of tyramine-evoked norepinephrine release from sympathetic nerves dates back to the 1960s. In irises incubated with D,L - 3H -norepinephrine, tyramine increased release of radioactivity into the medium, and the radioactivity was due exclusively to norepinephrine (33). In the same study, absence of ionized calcium in the incubation medium produced a slight augmentation of tyramine-induced release of radioactivity, leading to the inference that tyramine-induced norepinephrine release from sympathetic nerves is not dependent on extracellular ionized calcium. Electrical stimulation of bovine splenic nerves evokes dose-related release of dopamine- β -hydroxylase (which is confined to storage granules in sympathetic nerves), whereas tyramine does not (34). In the perfused guinea pig heart, tyramine evokes calcium-independent release of norepinephrine and its neuronal metabolite, dihydroxyphenylglycol (35).

The results of this study have potential implications for clinical neuropharmacology and pathophysiology. Drugs increasing sympathetic outflow or decreasing neuronal reuptake would be expected to be associated with increased plasma concentrations of both catecholamines; those releasing NE by nonexocytotic mechanisms would be associated with larger DA than NE responses, and those decreasing exocytosis would be associated with low plasma concentrations of both catecholamines. Disorders involving decreased activity of the vesicular monoamine transporter or DBH would be expected to entail lower plasma NE than DA, as has been reported in DBH deficiency (36) and in Menkes disease (37). In POTS, increased plasma concentrations of both DA and NE are consistent with increased overall sympathetic nerve traffic, decreased neuronal reuptake, or both (38, 39). Finally, all patients with familial dysautonomia have increased plasma DA:NE ratios (40), which, given the present findings, suggests augmented release from vesicles containing newly synthesized NE. Enhanced understanding about sources and meaning of plasma DA concentrations, in the context of data about plasma concentrations of other catechols, may provide biomarkers with which to diagnose and track progression of diseases and monitor effects of treatment.

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