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Dopamine Contamination of Infused Tyramine

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Clinical assessments of autonomic function often include tyramine (TYR) infusion. After uptake of TYR by sympathetic nerves via the cell membrane norepinephrine (NE) transporter and translocation of axoplasmic TYR into vesicles, NE exits the vesicles. Some of the NE enters the extracellular fluid and occupies adrenoceptors on cardiovascular smooth muscle cells, increasing blood pressure. A small proportion reaches the circulation, so that plasma NE concentrations increase (1–3).

Jacob et al. (4) reported an ~50-fold mean increase in plasma dopamine (DA) concentrations during intravenous TYR infusion, associated with "paradoxical" forearm vasodilation. We also noted high plasma DA concentrations in healthy volunteers during TYR infusion (5). When we assayed the catechol contents of the TYR infusate dispensed by our pharmacy [1 g/L (6.5 mmol/L)], we found that the infusate contained ~50 μmol/L DA, corresponding to 0.7% contamination (6). Whether during TYR infusion such contamination could actually raise plasma DA concentrations was unclear, as was whether, while stored as is customary in solution at 4 °C, TYR could be converted to DA. The present study examined these possibilities.

Solutions of TYR for infusion were prepared by the NIH Pharmaceutical Development Service, using TYR hydrochloride (Sigma) dissolved in sterile water, with pH ~9. The solutions were stored either in a refrigerator at 4 °C or in an ultra-low temperature freezer at −70 °C for up to 9 months and then were assayed for catechol content, including DA.

Infusate and arterial plasma concentrations of catechols were assayed (7), and hemodynamics were assessed before and during intravenous TYR infusion [6.5 μmol (1 mg)/min] in a total of 34 adults who underwent TYR infusion as part of autonomic function testing. Of the 34 participants, 6 were healthy volunteers, 13 were patients with chronic orthostatic intolerance, 9 had neurogenic orthostatic hypotension, 5 had undergone thoracic sympathectomy, and 1 had an undiagnosed movement disorder. All gave informed written consent before participating in the study protocols, which were approved by the Intramural Research Board of the National Institute of Neurological Disorders and Stroke.

Blood samples were drawn after at least 15 min with the person supine. For arterial sampling, a brachial catheter was placed percutaneously after local anesthesia of the overlying skin. Arm venous blood was drawn through an indwelling intravenous catheter.

Forearm blood flow was measured by venous occlusion strain gauge plethysmography (Hokanson). The circulation of the hand was not excluded. Forearm vascular resistance was calculated from the ratio of mean arterial pressure to forearm blood flow. Blood pressure was

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monitored continuously, either via the arterial catheter or noninvasively by a finger oscillometric (Finometer or Portapres; TNO) or radial tonometric (Colin) device. Cardiac stroke volume was measured via impedance cardiography (Cardiodynamics), a noninvasive method we had validated previously (8).

Neurochemical and hemodynamic data were analyzed by linear regression and dependent-means *t*-tests. Values are reported as the mean (SE). A *P* value <0.05 defined statistical significance.

All of the 29 TYR infusates assayed after infusion into humans contained DA. During ~9 months of refrigerated (4 °C) storage of TYR solutions in the dark, DA in the infusate increased exponentially, from <25 to >00 nmol/L (*r* = 0.99; *P* <0.0001; Fig. 1A). In contrast, the DA concentration in TYR infusate stored frozen at −70 °C remained unchanged.

The neurochemical and hemodynamic results were unrelated to diagnosis. Below a DA concentration of 50 nmol/L in the infused TYR solution, there was no relationship between the increment in arterial plasma DA and the infusate DA concentration (Fig. 1B). Above an infusate DA concentration of 50 nmol/L, arterial plasma DA increased with the infusate DA concentration (*r* = 0.91 overall; *P* <0.001).

Infusion of TYR solutions with DA concentrations ≤50 nmol/L was associated with small increases in arterial plasma DA, larger increases in plasma NE, and even larger increases in plasma dihydroxyphenylglycol (DHPG; Table 1). TYR infusion also increased mean arterial pressure (*t* = 5.6; *P* <0.0001) and cardiac stroke volume (*t* = 4.2; *P* = 0.001) but did not affect the venous-arterial difference in plasma NE, heart rate, or total peripheral or forearm vascular resistance.

Increments in arterial plasma NE during infusion of uncontaminated TYR were correlated with increments in plasma DA (*r* = 0.70; *P* = 0.0003) and with increments in plasma DHPG (*r* = 0.45; *P* = 0.03). Increments in cardiac stroke volume were strongly positively correlated with those in arterial plasma NE (*r* = 0.83; *P* = 0.003).

TYR hydrochloride [1 g/L (6.5 mmol/L)] assayed immediately after being dissolved in deionized water contained ~42 nmol/L DA. During incubation at 37 °C in a water bath, the DA concentration decreased with a half-time of 224 min. When TYR hydrochloride was dissolved in tap water, the initial DA concentration was already low, at ~1300 ng/L, or 9 μmol/L.

The results of this study show that TYR in aqueous solution undergoes nonenzymatic oxidation to DA. Over a period of 9 months of refrigerated storage of TYR solutions, the DA concentration increased exponentially, by ~25-fold. Over a similar time period, TYR in solutions stored at −70 °C the DA concentrations did not change.

Previously reported high plasma DA concentrations and forearm vasodilation during infusion of TYR solutions in humans therefore could have resulted from contamination of the infusates by DA produced during prolonged storage of TYR solution at 4 °C. In agreement with this notion, during intravenous TYR infusion into humans, the increment in the arterial plasma concentration of DA varied directly with the concentration of DA in the infusate. In one person, after receipt of TYR that had been stored for 9 months at 4 °C, the arterial plasma DA concentration exceeded 3 nmol/L, ~100 times higher than the typical concentration. The same person had a 30% decrease in forearm vascular resistance during the infusion. After making this observation, we did not think ethically or scientifically sound to administer such con taminated TYR. Subsequently, we used TYR solutions stored at −70 °C.

From the results depicted in Fig. 1, the observed plasma DA increment in the study of Jacob et al. [650 ng/L (4 nmol/L) (4)] would have corresponded to ~2% contam ination of the TYR infusate—well within quality-control limits. Infusion of relatively uncontaminated TYR in present study produced much smaller increments plasma DA that were not associated with forearm vaso dilation. In healthy humans, intravenous DA infusion ($0.5–2.0 \, \mu g \cdot kg^{-1} \cdot min^{-1}$) also does not affect forearm vascular resistance (9).

One might expect that forearm vascular resistance would increase during TYR infusion, because of local release. In humans, however, the pressor response intravenous TYR reflects cardiac stimulation, not systemic vasoconstriction (10), as confirmed in the present study Apparently, TYR releases NE from sympathetic nerves the myocardium, where they are much more abundant than in skeletal muscle. If TYR released NE from sympa thetic nerves in the forearm, then the venous-arterial difference for plasma NE in the arm should have creased; instead, the small arm venous-arterial difference for plasma NE did not change. Meanwhile, increments cardiac stroke volume were strongly positively correlated with increments in arterial plasma NE.

During infusion of uncontaminated TYR, arterial plasma concentrations of DA, NE, and DHPG all creased in a correlated manner, with the largest incre ments in plasma DHPG and the smallest in plasma One can understand these results in terms of TYR displac ing NE from vesicular stores into the axoplasm, where NE would undergo oxidative deamination to an aldehyde intermediate, followed by reduction of the aldehyde DHPG (1,11,12). A small amount of vesicular NE would enter the extracellular fluid and occupy postsynaptic cardiovascular adrenoceptors or reach the plasma, explaining the strong positive correlation between increments in cardiac stroke volume and in arterial plasma NE concentrations. An even smaller amount of DA would enter the plasma, reflecting vesicular DA not yet converted to NE.

From the results of incubating TYR solutions at 37 °C, we inferred that DA formed from oxidation of TYR is itself rapidly oxidized, particularly in tap water solutions. Iron ions in tap water would be expected to catalyze oxidation of DA to dopaminechrome (13).

Before the discovery of tyrosine hydroxylase as the rate-limiting step in biosynthesis of catecholamines, researchers considered the possibility that endogenous catecholamines might arise from autooxidation of tyrosine. In the body, the rate of 3,4-dihydroxyphenylalanine (DOPA) formation by autooxidation of tyrosine was found to be negligible compared with the rate of formation by enzymatic catalysis. The present findings serve as a reminder that DA can be synthesized ex vivo by nonenzymatic oxidation of TYR. For both scientific and ethical reasons, investigators who carry out clinical studies involving TYR administration should assay DA concentrations to exclude contamination of the infusate. From a practical point of view, to minimize this contamination, we recommend that TYR be dissolved in deionized water and stored frozen at −70 °C.

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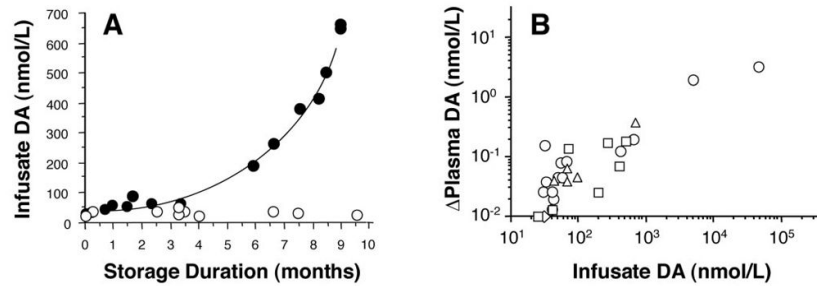
Fig. 1.
Concentrations of DA in TYR solutions as a function of duration of storage at 4 °C (●) and −70 °C (○; *A*), and increment in arterial plasma DA concentration as a function of infusate DA concentration in humans undergoing intravenous infusion of TYR (*B*).
(*A*), equation for the line: $y = 38^{(0.31x)}$; $r = 0.99$. (*B*), ○, patients with chronic orthostatic intolerance; □, patients with chronic autonomic failure; △, patients status post bilateral thoracic sympathectomies for hyperhidrosis; ◇, healthy volunteer.

Table 1

Neurochemical and hemodynamic values during supine rest and after 10 min of TYR infusion (infusate DA ≤50 nmol/L).

Value	Baseline[a]	TYR infusion[a]
DA_a,[b] ng/L	0.07 (0.01) [n = 12]	0.10 (0.02)[c] [n = 12]
NE_a, ng/L	1.17 (0.19) [n = 13]	1.67 (0.32)[c] [n = 13]
NE_v, ng/L	1.09 (0.26) [n = 9]	1.77 (0.49)[c] [n = 11]
AV diff NE, ng/L	0.02 (0.08) [n = 9]	0.17 (0.36) [n = 10]
$DHPG_a$, ng/L	4.30 (0.37) [n = 13]	7.82 (0.76)[d] [n = 13]
$DHPG_v$, ng/L	4.42 (0.78) [n = 9]	7.49 (1.18)[c] [n = 10]
Heart rate, beats/min	67 (5) [n = 10]	63 (4) [n = 10]
Stroke volume, mL	82 (7) [n = 10]	94 (8)[e] [n = 10]
Cardiac output, L/min	5.3 (0.3) [n = 10]	5.7 (0.4) [n = 10]
Total peripheral resistance, mmHg · min/L	1694 (171) [n = 10]	1756 (172) [n = 10]
MAP, mmHg	108 (4) [n = 23]	119 (5)[d] [n = 23]
FBF, mL · min^{-1} · dL^{-1}	4.2 (0.4) [n = 23]	4.1 (0.3) [n = 23]
FVR units	32 (5) [n = 23]	34 (5) [n = 23]

[a] Values are the mean (SE), with the numbers in brackets indicating the numbers of individuals.

[b] DA_a, arterial plasma DA; NE_a, arterial plasma NE; NE_v, arm venous plasma norepinephrine; AV diff, venous-arterial difference; $DHPG_a$, arterial plasma DHPG; MAP, mean arterial pressure; FBF, forearm blood flow; FVR, forearm vascular resistance.

[c–e] Significant difference from baseline:

[c] $P < 0.05$;

[d] $P < 0.001$;

[e] $P < 0.01$.