Production of cysteinyl-dopamine during intravenous dopamine therapy

JULIA L. MAGNAY, JEFF TONG, ROSA DRANGOVA, and ANDREW D. BAINES

Department of Laboratory Medicine and Pathobiology, University of Toronto and The University Health Network, Toronto, Ontario, Canada

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Background. Oxidized dopamine rapidly forms thiol-conjugates with −SH groups on cysteine, glutathione, and proteins. We used cysteinyl-dopamine production as an index of thio-ester production during intravenous dopamine treatment of critically ill patients.

Methods. Cysteinyl-dopamine and catecholamines were measured by high-performance liquid chromatography with electro-chemical detection. The production of cysteinyl-dopamine by purified human neutrophils was measured using dopamine (1 μmol/L) and cysteine (1 mmol/L) concentrations similar to those found during dopamine treatment. To examine the impact of endotoxic shock on cysteinyl-dopamine production, anesthetized rats were given dopamine (12 to 15 μg/kg/min intravenously) with or without endotoxin (50 mg/kg intravenously).

Results. In vitro, neutrophils converted 26% of dopamine to cysteinyl-dopamine (30 min at 37°C). Activating neutrophils with zymogen increased dopamine consumption from 26 to 68%, but only 36% appeared as cysteinyl-dopamine. The remainder may have been oxidized to other cysteinyl derivatives. Endotoxin increased cysteinyl-dopamine in rat plasma from 2.5 nmol/L (range <0.2 to 11) to 9.7 nmol/L (range <0.3 to 31, $P = 0.1$). After four hours, with or without endotoxin, cysteinyl-dopamine was <0.3 nmol/L in cerebrospinal fluid. In the plasma of eight patients receiving dopamine (6 to 20 μg/kg/min for 1 to 3 days), dopamine was 0.5 to 9.9 μmol/L, and cysteinyl-dopamine was 48 to 1660 nmol/L. Cysteinyl-dopamine was 4.3 to 22.6% of dopamine and correlated with leukocyte count ($r^2 = 0.388$, $P = 0.099$).

Conclusions. A significant fraction of exogenously administered dopamine reacts with −SH groups of cysteine and probably also with −SH groups on peptides and proteins. During brief dopamine treatment of endotoxic shock in rats, neither dopamine nor cysteinyl-dopamine crossed the blood–brain barrier.

Dopamine is the most readily oxidized and in vitro the most cytotoxic of the naturally occurring catecholamines. Concentrations of 10^{-5} mol/L destroy a variety of cells in culture [1–3]. Oxidation of dopamine produces a quinone that reacts avidly with nucleophilic sulfhydryl (SH) groups on cysteine and glutathione to form the SH conjugates 5-S-glutathionyl-dopamine (GSH-DA) and 5-S-cysteinyl-dopamine (CYS-DA) [4, 5]. Conjugation of dopamine-quinone with −SH occurs 1000-fold faster than production of aminochrome by internal cyclization [6]. Formation of covalent bonds between dopamine-quinone and nucleophilic −SH groups on proteins may disrupt protein structure and enzyme activity. In addition, the oxidation of CYS-DA produces a variety of neurotoxic products such as dihydrobenzothiazines and benzothiazines [7–9]. These substances are lethal when injected into the brains of mice [10].

Conditions that produce cytotoxic dopamine thiol-conjugates may exist when dopamine is used to treat patients with septic or cardiovascular shock. Intravenous dopamine infusion for hours to days raises plasma unconjugated dopamine concentrations to the micromolar range. Free radicals generated by activated neutrophils [11] or from reperfusion after ischemia [11–13] could increase dopamine oxidation and the production of dopamine thiol-conjugates. This hypothesis was tested by measuring CYS-DA production in vitro after incubation of dopamine with activated neutrophils, by measuring CYS-DA and GSH-DA concentrations in rats after endotoxin injection and subsequent dopamine infusion, and by measuring plasma CYS-DA and GSH-DA concentrations in critically ill patients who were receiving dopamine infusions.

METHODS

Materials

Dopamine hydrochloride, glutathione, L-cysteine, tyrosinase (from mushroom 1000 U/mg solid), acivicin (α-amino-3-chloro-4,5-dihydro-5-isoxazole-acetic acid),

and endotoxin lipopolysaccharide from *Escherichia coli*—serotype 055:B5—were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Sodium acetate, sodium octane sulfonate, and ethylenediaminetetraacetic acid (EDTA) were obtained from BDH Inc. (Toronto, Ontario, Canada). Citric acid was obtained from JT Baker Chemical Co. (Phillipsburg, NJ, USA). Acetonitrile high-performance liquid chromatography (HPLC) grade and sodium phosphate were purchased from Caledonian Laboratories Ltd. (Georgetown, Ontario, Canada). Dibutylamine was obtained form Aldrich Chemical Co. (Milwaukee, WI, USA).

Catecholamine and 5-S-cysteinyl dopamine analysis

Catecholamine analysis was performed using HPLC with electrochemical detection. To increase analytical specificity, two different HPLC systems were used, with different detector voltages, mobile phase composition, and pH. The JCL6000 system (Jones Chromatography USA Ltd., Lakeword, CO, USA) used a guard column filled with C18 pellicular packing (Alltech Associates, Deerfield, IL, USA) and a 3 μm packing LC-18 Supelcosil 4.6 × 75 mm column (Supelco Canada Ltd., Oakville, Ontario, Canada). The mobile phase was 50 mmol/L sodium acetate, 22.4 mmol/L citric acid, 1.35 mmol/L EDTA, 3.75 mmol/L sodium octane sulfonate, 0.01% (wt/vol) dibutylamine, and 4.5% (vol/vol) acetonitrile, adjusted to pH 4.3 to 4.4 with acetic acid, filtered (4.5 μm), and degassed. The flow rate was 0.7 mL/min at an operating pressure of 9.4 Mpa (1200 psi). The HPLC was connected to a Model 5100A coulometric detector consisting of three coulometric cells in series (ESA Inc., Bedford, MA, USA). The conditioning cell was set at 0.15 V. Analytical cell electrodes I and II were set at 0.2 and −0.38 V, respectively. Data analysis was performed by a JCL6000 Chromatography Data System (Jones Chromatography USA Ltd.) connected to electrode II.

The Millennium HPLC system used a Zorbax C18 column with an online filter. The mobile phase consisted of 100 mmol/L sodium phosphate, 0.27 mmol/L EDTA, 0.92 mmol/L sodium octane sulfonate, and 3.0% (vol/vol) acetonitrile and was adjusted to pH 3.6 with phosphoric acid and degassed. The flow rate was 1.0 mL/min at an operating pressure of 9.4 Mpa (1200 psi). The HPLC was connected to a Model Coulochem II coulometric detector consisting of four coulometric cells in series (ESA Inc.). The guard cell was set at 0.15 V. Electrodes I, II, III, and IV were set at 0.0, 0.0, 0.20 and −0.38 V, respectively. Data analysis was performed by a Millennium 2020 chromatography manager (Waters Associates Inc., Millford, MA, USA).

Catecholamines and their metabolites, including CYS-DA and GSH-DA, were extracted from plasma and urine with alumina. Catecholamines were released from the alumina by the addition of 0.15 mol/L $HClO_4$ in a phosphate mobile phase. To quantitate the total catecholamines (free and conjugated), urine was adjusted to a pH of <2 with 6 mol/L HCl and hydrolyzed for 60 minutes at 95°C before extraction. The detection and quantitation of CYS-DA were satisfactory using both systems. Norepinephrine and GSH-DA had virtually identical retention times when analyzed by the JCL6000 system (about 5 min), but the peaks were well separated using the Millennium system (~6.5 and 40 min, respectively). Consequently, CYS-DA and GSH-DA were identified in biological samples by a comparison of retention times on both HPLC systems and by co-chromatography with standards. Recovery of all catecholamines with the exception of dihydroxyphenyl acetate (DOPAC) was between 65 and 85% following alumina extraction. The recovery of DOPAC was consistently approximately 50%. Interassay coefficients of variation were dihydroxyphenylglycol (DHPG) 6.0%, DOPA 3.1%, NE 2.8%, E 4.6%, DOPAC 8.5%, dopamine 7.2%, CYS-DA 3.3%, and GSH-DA 2.8% ($N = 10$). The detection limit of both CYS-DA and GSH-DA (assuming 1.0 mL of undiluted specimen was processed) was 0.1 nmol/L. Linearity was confirmed up to 200 nmol/L. CYS-DA was stable to hydrolysis, but 99% of GSH-DA was destroyed by the procedure; therefore, we were unable to measure conjugated GSH-DA. Both GSH-DA and CYS-DA were stable at acid pH ($HClO_4$, pH 1.1) and room temperature. Standard solutions of GSH-DA and CYS-DA were stored at −70°C in 2 mol/L HCl.

To produce 5-S-cysteinyl dopamine, dopamine-HCl (20 mg, 0.105 mmol) and L-cysteine (35 mg, 0.22 mmol) were incubated with mushroom tyrosinase (10 mg, 10,000 units) in 50 mL of 0.5 mol/L phosphate buffer (pH 6.5) [4]. After 60 minutes, a dopamine peak was no longer detectable by HPLC, and the enzymatic reaction was stopped by adding 5 mL of 4 mol/L $HClO_4$. CYS-DA was isolated by elution with 2 mol/L HCl from a Dowex-50 W-X4 (200 to 400 mesh hydrogen form) column. A dark purple residue with ultraviolet absorption maxima at 255 and 292 nm was obtained. Nuclear magnetic resonance (NMR) spectral analysis confirmed that the compound was CYS-DA. To produce 5-S-glutathionyl dopamine, dopamine HCl (40 mg, 0.21 mmol) and glutathione (700 mg, 2.28 mmol) were incubated with mushroom tyrosinase (10 mg, 10,000 U) in 25 mL of 0.1 mol/L ammonium acetate buffer (pH 5.8) for five hours [5]. A brown substance was obtained was eluted with 2 mol/L HCl from a Dowex column. UV maxima were at 255 and 292 nm [4, 5], and NMR spectral analysis confirmed that the compound was GSH-DA. S-(N-acetylcysteinyl)-dopamine was synthesized by electrolysis of dopamine to produce dopamine-o-quinone followed by addition of N-acetyl cysteine [8]. The major product of this reaction has been identified by Shen, Zhang, and Dryhurst as 5-S-

(N-acetylcysteinyl)-dopamine [8]. The reaction mixture was extracted with alumina and separated with HPLC. The major unidentified peak appeared midway between the dopamine and CYS-DA peaks on the Millennium system.

Incubation of 5-S-cysteinyldopamine with activated neutrophils

Activated neutrophils were prepared from citrated blood obtained from human volunteers. All reagents were sterile. Blood was centrifuged for 20 minutes at $175 \times g$, and the plasma was removed down to the buffy coat. This plasma was layered onto a button of 90% percoll and centrifuged at $1000 \times g$ to produce platelet-poor plasma. After removal of the plasma, the remaining cells were resuspended with 5 mL of 6% dextran T-500 in 0.9% wt/vol saline, made up to 50 mL with saline, and allowed to stand for 30 minutes. The supernatant was then centrifuged at $112 \times g$ for six minutes at room temperature. The resulting pellet was resuspended in 2 mL of platelet-poor plasma and underlayered with 51 and 42% percoll gradients. The cells and percoll were centrifuged at $180 \times g$ for 10 minutes, and then the neutrophil-enriched intermediate layer was washed in Hank's solution containing 110 U/L heparin. The cells were used immediately for further experiments; 10^7 neutrophils were incubated in 1 mL of Hank's solution with 1 mol/L cysteine and 1 μmol/L dopamine with or without opsinized zymogen (25 μL) to activate them. These concentrations of cysteine and dopamine are typically encountered in the plasma of septicemic patients undergoing dopamine therapy. After 30 minutes at 37°C, the reaction was stopped with 40 μL of 70% HClO$_4$. Catecholamines were measured in the supernatant after alumina extraction. All assays were performed in triplicate.

Dopamine infusion into endotoxin-shocked rats

Male Wistar rats (310 to 350 g) were used to test the effect of endotoxic shock on the production of GSH-DA and CYS-DA during dopamine infusion. Twenty-four animals were anesthetized (50 mg/kg pentobarbitol IP), catheterized, and infused for 60 minutes with 0.9% wt/vol saline (3 mL/hour). Systolic blood pressure was monitored continuously through a cannula in the carotid artery during the study. Groups 1 and 2 were given an IV bolus injection of *Escherichia coli* endotoxin—serotype 055:B5 (50 mg/kg body weight)—in 500 μL saline. Groups 2 and 4 were given saline. After five minutes, groups 1 and 3 were infused with dopamine (12 μg/kg/min) for 30 minutes. Groups 2 and 4 were infused with saline. Blood (6 mL) was then taken for catecholamine measurement. Another group of eight rats was anesthetized with inactin (100 mg/kg, IP) and prepared for collection of cerebrospinal fluid (CSF). A catheter was implanted in each rat's cisterna magna following the

method of Huang et al [14]. The rats were then infused with dopamine (15 μg/kg/min) for four to five hours. Four of the rats also received 50 mg of endotoxin at the beginning of the dopamine infusion. CSF was collected during the final two hours of the infusion. An additional six anesthetized rats were infused with dopamine for five hours without the cisternal catheter, and three of these rats also received endotoxin. Urine was collected during the fourth hour of the dopamine infusion. Samples were immediately centrifuged, separated into 1 mL aliquots, and stored at −70°C until analysis.

Cysteinyldopamine in plasma from patients receiving intravenous dopamine

After obtaining informed consent or surrogate informed consent, heparinized blood samples (7 mL) were collected from eight critically ill patients who were receiving intravenous dopamine (6 to 20.5 μg/kg/min) at the Toronto General Hospital Division of the University Health Network. The samples were centrifuged immediately at 4°C and stored at −70°C until analysis. This study was approved by the UHN Research Ethics Board.

Statistics

Data were calculated using SigmaStat for Windows (Jandel Scientific Software, San Rafael, CA, USA). A comparison of catecholamine results between the groups was assessed by Kruskal–Wallis one-way analysis of variance (ANOVA) on ranks, followed by the Student Newman–Keuls multiple comparison procedure to isolate the groups or groups that differed from the others. Statistical significance was considered at P values of ≤ 0.5.

RESULTS

Incubation of 5-S-cysteinyldopamine with activated neutrophils

In the absence of neutrophils, 26 nmol/L (2.6%) of dopamine were converted to CYS-DA (Fig. 1). The addition of neutrophils to the incubation mixture increased the conversion of dopamine to CYS-DA by 10-fold to 255 nmol/L (26% of initial dopamine concentration). After neutrophil activation by zymogen, 680 nmol/L of dopamine disappeared; however, the CYS-DA concentration only increased to 364 nmol/L (36% of initial dopamine concentration). Therefore, in an environment of increased free radical production, the disappearance of dopamine substantially exceeded the measured appearance of CYS-DA. Apart from dopamine and CYS-DA, no other metabolites were detected, except a minor unidentified peak that was possibly a CYS-DA oxidation product. This peak was also observed in the CYS-DA standard.
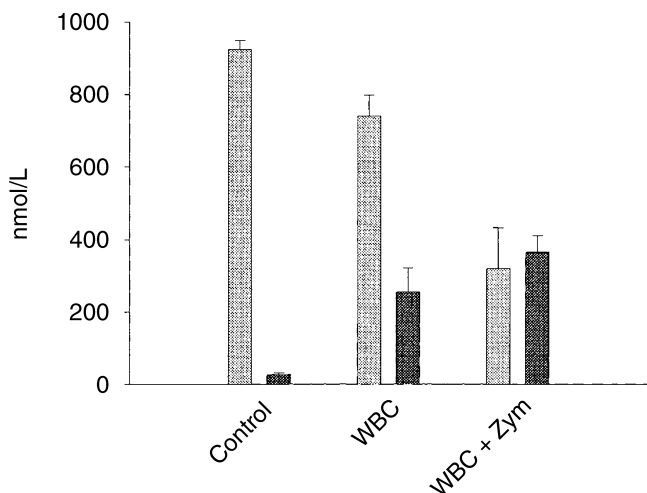
Fig. 1. Conversion of dopamine to 5-S-cysteinyl dopamine (CYS-DA; ■) by activated neutrophils. Dopamine (DA; 1 μmol/L, ▨) was incubated with cysteine (Cys 1 mmol/L) for 30 minutes alone or in the presence of 10^7 neutrophils (WBC)/mL. Zymogen was added to activate the neutrophils. Neutrophils significantly increased CYS-DA production and disappearance of dopamine ($P = 0.03$, ANOVA). Activating the WBC with zymogen increased dopamine removal ($P = 0.03$) but did not significantly increase CYS-DA production ($N = 3$).

Dopamine infusion into endotoxin-shocked rats

Systolic blood pressure was between 100 and 145 mm Hg during the one-hour stabilization period prior to endotoxin or saline injection. During the 10 minutes following endotoxin injection, blood pressure decreased to 30% of baseline values in rats that received dopamine and those that did not. Blood pressure increased over the next 20 minutes to approximately 60% of baseline values and remained there for up to 4.5 hours. Blood pressure did not alter significantly from baseline in the groups that received saline or dopamine without endotoxin throughout the experiment. Hematocrit levels increased significantly ($P < 0.001$) in rats that received endotoxin but did not change in the others.

Intravenous dopamine, by itself within 30 minutes, raised plasma concentrations of dopamine and CYS-DA as well as the metabolites, DHPG and DOPAC (Table 1). Endotoxin, by itself, increased DHPG, DOPA, norepinephrine, epinephrine, and DOPAC, but not CYS-DA concentration. CYS-DA concentrations were not normally distributed in rats that received dopamine alone. High CYS-DA concentrations were associated with transient incomplete anesthesia in two rats that did not receive endotoxin. The superimposition of intravenous dopamine on endotoxic shock increased plasma CYS-DA, but because of the wide scatter of results the median concentration (9.7 nmol/L), was not significantly higher than with dopamine alone (2.3 nmol/L, $P = 0.13$, Mann–Whitney rank sum test). The combination of endotoxin and dopamine increased the concentration of norepi-

nephrine above that seen with endotoxin or dopamine alone. CYS-DA was undetectable in plasma of rats that did not receive dopamine. GSH-DA was not detected in any of the plasma samples. Similar results were obtained after five hours of dopamine infusion (Table 2). At that time, plasma dopamine levels were lower, but the difference was not significant.

An unknown peak with the same retention time as the major product from the reaction of dopamine-o-quinone with N-acetylcysteine [8] appeared in chromatographs of plasma from rats that received dopamine. The unknown peak was not found in urine or in plasma from control rats that did not receive dopamine. The peak height was similar to that of CYS-DA. In rats that received dopamine, endotoxin did not alter the mean peak height of the unknown substance (67 ± 31 μV after 30 min dopamine and 76 ± 50 μV after dopamine plus endotoxin). After four to five hours, the peak heights were 160 ± 115 and 85 ± 63 μV. In samples collected after 30 minutes of dopamine infusion, the unknown peak tended to be inversely correlated with the CYS-DA peak ($P = 0.06$ in the group that received endotoxin and $P = 0.14$ in the group that received dopamine without endotoxin). There was no correlation of the unknown peak with CYS-DA dopamine in the plasma collected after four to five hours of infusion.

Urine excretion decreased to virtually zero for the first hour following the injection of endotoxin; therefore, we only examined the results for rats that received four to five hours of dopamine infusion. In preliminary studies with dopamine infused rats, we found that urine free CYS-DA was $23 \pm 10\%$ of total CYS-DA. Urine volume was too small to measure total as well as free CYS-DA in the endotoxin-treated rats. Endotoxin increased the excretion of norepinephrine and epinephrine during dopamine infusion (Table 3), but did not significantly alter the excretion of dopamine or CYS-DA. Urinary-free CYS-DA excretion did not correlate with plasma dopamine or CYS-DA, but was correlated with plasma norepinephrine ($P = 0.02$) and epinephrine ($P = 0.007$) in the group that received both endotoxin and dopamine.

Cerebrospinal fluid was collected during the final two hours of four- to five-hour dopamine infusions (Table 4). CYS-DA was below the level of detection in all samples. Endotoxin did not increase the CSF concentrations of dopamine or its metabolites.

Catecholamine analysis of plasma from patients receiving intravenous dopamine

Using both HPLC systems, CYS-DA was identified in plasma from eight patients that were receiving dopamine for a variety of acute critical conditions (Table 5). The CYS-DA concentration was related to plasma dopamine concentration ($r^2 = 0.97$). Plasma CYS-DA as a percentage of dopamine tended to increase with white blood cell

Table 1. Plasma catecholamines metabolites after 30 minutes of dopamine infusion with and without endotoxin

Group	DHPG	DOPA	E	NE	DOPAC	DA	CYS-DA
1 Endotoxin	18[ab]	5.1[a]	27[ac]	4.6[abc]	291[ab]	330[ab]	9.7[ab]
Dopamine	(14–21)	(4–6)	(3–40)	(3–7)	(228–364)	(200–389)	(0.2–14)
2 Endotoxin	11[a]	7.0[ac]	24[ac]	2.3[a]	10[a]	2	<0.4
Saline	(11–16)	(6–10)	(9–39)	(2–9)	(6–15)	(1–8)	
3 Control	12[a]	7[a]	0.9[a]	2.1	242[ab]	282[ab]	2.3[ab]
Dopamine	(11–14)	(4–9)	(0.7–0.9)	(1–3)	(124–349)	(165–392)	(0–2.6)
4 Control	6	2.0	<0.2	1.7	4	0.2	<0.3
	(5.6–6.4)	(2–9)		(1–2)	(3–5)	(0.1–6)	

Median concentration is in nmol/L with 25 to 75% confidence limits. Abbreviations are: DHPG, dihydroxy phenylglycol; DOPA, dihydoxyphenylalanine; E, epinephrine; NE, norepinephrine; DA, dopamine; CYS-DA, 5-S-cysteinyl-dopamine. Endotoxin (50 mg/kg) was injected into groups 1 and 2, while groups 1 and 3 received dopamine (12–15 μg/kg·min IV). Blood was collected after 30 minutes of dopamine infusion with or without endotoxin or saline control. One-way ANOVA on ranks $P < 0.05$. $N = 5$ to 9 in each group.
[a] Significantly greater than group 4 ($P < 0.05$)
[b] Significantly greater than group 2 ($P < 0.05$)
[c] Significantly greater than group 3 ($P < 0.05$)

Table 2. Plasma catecholamines metabolites after 300 minutes of dopamine infusion with and without endotoxin

	DHPG	DOPA	E	NE	DOPAC	DA	CYS-DA
Endotoxin	20[a]	4	48	14[b]	259	80	5
Dopamine	(17–22)	(4–6)	(6–55)	(10–16)	(183–324)	(53–179)	(1.5–16)
Control	15	2	6	3	257	131	2.6
Dopamine	(12–16)	(1–4)	(0.3–39)	(1–4)	(191–381)	(62–427)	(0–7.8)

Median concentration is in nmol/L with 25 to 75% confidence limits. Abbreviations are: DHPG, dihydroxy phenylglycol; DOPA, dihydoxyphenylalanine; E, epinephrine; NE, norepinephrine; DA, dopamine; CYS-DA, 5-S-cysteinyl-dopamine. Endotoxin (50 mg/kg) was injected into one group ($N = 7$) and the other group received only saline ($N = 6$). Both groups received dopamine (15 μg/kg/min IV). Blood was collected after 300 minutes of dopamine infusion with or without endotoxin or saline control. P values are for an unpaired t test with DHPG and NE. Other metabolites were not significantly different.
[a] $P = 0.02$, [b] $P = 0.03$

Table 3. Effect of endotoxin on urine catecholamine excretion during the fourth hour of dopamine infusion

	DHPG	NE	E	DOPAC	DA	CYS-DA
Endotoxin						
Dopamine	11 ± 2	28 ± 4[a]	41 ± 9[b]	2240 ± 400	3360 ± 550	9.5 ± 3
Control						
Dopamine	13 ± 1	13 ± 3	6 ± 3	1590 ± 350	3030 ± 800	4.5 ± 2

Mean ± SE excretion rates pmol/min. Dopamine was infused at 15 μg/kg/min into 5 rats. Urine was collected during the fourth hour of dopamine infusion, another 7 rats received dopamine plus endotoxin 50 mg/kg. P values are for an unpaired t test. Abbreviations are in the Table 2 legend.
[a] $P = 0.03$, [b] $P = 0.02$

Table 4. Catecholamine metabolites in cerebrospinal collected during dopamine infusion with and without endotoxin

	DHPG	DOPA	NE	DOPAC	DA	CYS-DA
Endotoxin						
Dopamine	21 ± 1	1.2 ± 0.2	2.5 ± 1	152 ± 31	7 ± 5	<0.2
Control						
Dopamine	18 ± 2	1.6 ± 0.2	2.1 ± 0.7	106 ± 26	4 ± 1	<0.3

Data are means ± SE; concentration is in nmol/L. Dopamine was infused at 15 μg/kg/min into 4 rats. Another 4 rats received dopamine plus endotoxin 50 mg/kg. Cerebrospinal fluid (CSF) was collected during the second to fourth hour of dopamine infusion from both groups.

(WBC) count ($P = 0.099$; Fig. 2). Using stepwise forward regression analysis, the equation relating %CYS-DA/dopamine to WBCs was not significantly improved by adding plasma dopamine, dopamine dose, duration of treatment, or arterial pO_2. There was no significant relationship with the nature of the primary diagnosis. GSH-DA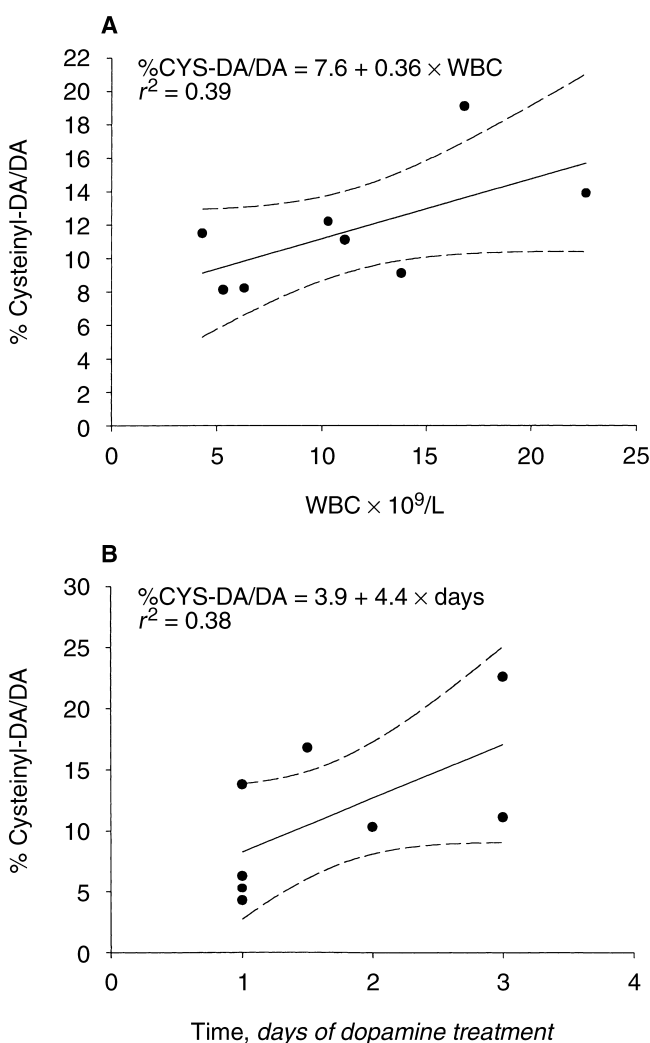 was not detected in any of the plasma samples. An unidenti-fied peak appeared midway between dopamine and CYS-DA in the patient's plasma. This unknown peak that may have been an N-acetyl cysteinyl-dopamine conjugate was smaller than the CYS-DA peaks.

In theory, dopamine should react with GSH as well as with cysteine. An explanation for the absence of GSH-DA from the rat and human samples was sought by adding

Table 5. 5-S-cysteinyl dopamine in plasma of patients receiving intravenous dopamine

Age/sex years	Condition	DA dose[a] $\mu g/kg \cdot min$	Days	Other vasoactive drugs	WBC × $10^9/L$	PO$_2$ mm Hg	Plasma DA $\mu mol/L$	Plasma CYS-DA nmol/L
60/M	Resp. arrest	8.6	3		13.9	106	0.5	113
51/M	Resp. arrest Lung transplant	11.2	1		9.1	86	0.9	124
74/M	Myocardial infarct	17.5	1	Levophed nitroglycerine	8.2	103	2.4	150
59/M	GI bleed alcoholic liver disease	6	3		11.1	72	0.7	78
68/M	Pancreatic abscess intra-abdominal bleed	6.7	2	Levophed	12.2	90	0.7	72
67/F	Resp. failure, lung resection for CA	15.6	1		11.5	119	1.4	60
82/M	Ruptured aortic aneurysm	8.5	1		8.1	115	0.9	48
48/F	Double lung transplant	20.5	1.5	Epinephrine, levophed, vasopressin	19.1	86	9.9	1,660

[a] Dopamine infusion at the time of sample collection

A

$$\%\text{CYS-DA/DA} = 7.6 + 0.36 \times \text{WBC}$$
$$r^2 = 0.39$$

% Cysteinyl-DA/DA

WBC × $10^9/L$

B

$$\%\text{CYS-DA/DA} = 3.9 + 4.4 \times \text{days}$$
$$r^2 = 0.38$$

% Cysteinyl-DA/DA

Time, *days of dopamine treatment*

Fig. 2. Unconjugated 5-S-cysteinyl dopamine (CYS-DA) expressed as a percentage of unconjugated dopamine (DA) in the plasma of patients undergoing dopamine treatment for acute illness. The percentage of CYS-DA/DA relative to the leukocyte count (WBC; *A*) is 7.6 + 0.36 × WBC, and relative to the duration of dopamine treatment (days; *B*) is %CYS-DA/DA = 3.9 + 4.4 × days, *r* = 0.38. The lines indicate the regression equation and 95% confidence limits.

Table 6. Effect of γ-glutamyl transpeptidase (GT) in human plasma on conversion of 5-S-glutathionyl dopamine (GSH-DA) to 5-S-cysteinyl dopamine (CYS-DA)

Minutes	High γGT plasma		High γGT plasma + acivicin	
	GSH-DA	CYS-DA	GSH-DA	CYS-DA
0	860	<0.15	860	<0.15
5	356	43	818	6
30	117	108	807	16

	Low γGT plasma		Low γGT plasma + acivicin	
0	860	<0.15	860	<0.15
5	656	<0.15	554	<0.15
30	390	<0.15	392	<0.15

Concentration is in nmol/L. Glutathionyl-dopamine (GSH-DA) was added to pooled plasma with high (270 U/L) or low (10 U/L) γ-glutamyl transpeptidase activity. Acivicin (1.5 mg/mL) was added to a paired sample to inhibit γ-glutamyl transpeptidase activity.

GSH-DA to pooled human plasma with high γ-glutamyl transpeptidase (γ-GT) activity (279 U/L). GSH dopamine (initially 860 nmol/L of plasma) was rapidly degraded and more than 80% disappeared after 30 minutes of incubation. The CYS-DA concentration increased from <0.15 nmol/L to 108 nmol/L after 30 minutes. Preincubation of the pooled plasma with acivicin to block γ-GT activity slowed the degradation of GSH-DA and CYS-DA production so that after 30 minutes, 808 nmol/L of GSH-DA remained and CYS-DA had only increased to 16 nmol/L (Table 6). Plasma with a low γ-GT concentration did not produce CYS-DA, although GSH-DA disappeared. In this plasma, the removal of GSH-DA was not sensitive to inhibition by acivicin.

DISCUSSION

Dopamine is readily oxidized to an o-quinone by neutrophils in the presence of oxygen at physiological pH [15]. The highly reactive dopamine-quinone follows three paths. It may (*1*) form conjugates with nucleophilic groups

such as the −SH on cysteine or proteins, (2) undergo intramolecular cyclization, or (3) be reduced back to the original catechol with the production of free oxygen radicals [1, 6, 16]. Dopamine-quinone is 1000 times more likely to react with nucleophilic −SH groups if they are available than to undergo intramolecular cyclization to produce an indoline [6]. The neutrophil experiments demonstrated the avidity of dopamine for −SH groups (Fig. 1). Neutrophils rapidly converted 26% of dopamine to CYS-DA when dopamine and cysteine concentrations were similar to those that exist in critically ill patients during treatment with dopamine (Table 5). Neutrophil activation doubled the rate of dopamine disappearance with a 10% increase in CYS-DA production. The small increase in CYS-DA is to be expected because CYS-DA is more easily oxidized than dopamine. Oxidized CYS-DA is converted to a variety of thiol-conjugates and their acetylated conjugates [7, 8, 10, 16].

The production of CYS-DA in anesthetized rats was measured after dopamine infusion for 30 to 300 minutes. At both 30 and 300 minutes, the unconjugated CYS-DA concentration was approximately 1% of the plasma dopamine concentration (Tables 1 and 2). After three to five hours of dopamine infusions, there was no detectable CYS-DA in CSF and CSF dopamine concentration was less than 10% of plasma concentration (Table 4). Conjugated CYS-DA was not measured in plasma but was found to be 23% of total CYS-DA in urine after acid hydrolysis. Dopamine-quinone reacts avidly with glutathione as well as with cysteine [6]; however, no GSH-DA was found in plasma or urine. The absence of GSH-DA is consistent with its rapid conversion to CYS-DA by γ-glutamyl transpeptidase (Table 6) [17] and to acetylated thiol-conjugates by cysteine conjugate acetyl transferase [8].

Endotoxemia did not significantly increase the conversion of infused dopamine to CYS-DA in plasma or urine. Interpretation of these experiments is confounded by sustained hypotension, which would have impaired renal metabolism and clearance of dopamine. Furthermore, oxidative destruction of both dopamine and CYS-DA may have increased as it did in the neutrophil experiments (Fig. 1) [16]. Variable degrees of oxidative stress could contribute to the wide range of plasma CYS-DA concentrations and the consequent lack of a significant difference between the control and endotoxin treated rats. Endotoxemia did not significantly increase the concentration of dopamine or CYS-DA in CSF, which suggests that at least for brief dopamine infusions with an intact blood–brain barrier, the risk of central nervous system exposure to dopamine and its conjugates is not great.

CYS-DA appeared in the plasma (Table 5) and urine (not shown) of critically ill patients undergoing treatment with dopamine. Concentrations of free CYS-DA were in the μmol/L range and ranged from 4 to 22.6%

of the plasma-free dopamine concentration. That neutrophils promote dopamine conjugation with cysteine is suggested by the correlation between the percentage of CYS-DA/dopamine and WBC count ($P = 0.099$; Fig. 2).

CYS-DA is only one product of many created by dopamine–quinone interaction with −SH groups [7–10, 18]. The presence of conjugated CYS-DA in rat urine suggests that an additional pool of conjugated CYS-DA was present in both rat and human plasma. Up to 70% of oxidized dopamine may bind to protein −SH groups [19]. Thus, we conclude that the free CYS-DA represents only a small portion of the total DA-SH conjugates formed by nucleophilic attack on oxidized dopamine in these patients.

During dopamine therapy, plasma dopamine concentrations are frequently greater than 1 μmol/L (Table 5), which is 1% of the LD_{50} for some cells in culture [20]. These concentrations of dopamine can alter protein structure and inactivate enzymes such as DNA polymerase [21] and other −SH dependent proteins [22]. Oxidation of CYS-DA produces dihydoxybenzothiazine, which is lethal when injected into the mouse brain [16]. Cysteinyl-DOPAC, derived from the reaction of cysteine with oxidized DOPAC, is highly toxic to cultured P19 cells and to hippocampal pyramidal neurons [9]. It is interesting to note that DOPAC concentrations increased 50-fold during the 30-minute dopamine infusion following endotoxin injection (Table 1); however, we did not look for cysteinyl-DOPAC specifically.

The concentration of CYS-DA may be viewed in two ways: as an index of dopamine–quinone interaction with −SH groups in general and as a reflection of the protective effect of cysteine and glutathione. Glutathione and cysteine, by forming thiol-conjugates with dopamine, reduce the binding of dopamine to protein −SH groups [23]. This protective action is shown by reduced dopamine-induced apoptosis in cultured PC12 cells [24]. However, while glutathione may reduce the reaction of dopamine with protein-SH groups, it does not necessarily protect against the cellular disruption produced by high concentrations of dopamine [23], and as previously noted, a number of the byproducts of GSH-DA and CYS-DA may be cytotoxic [7–10, 18].

We conclude that thioester formation contributes significantly to the metabolism of dopamine during dopamine therapy. Factors that promote oxidation of dopamine such as activated neutrophils will increase the formation of dopamine-thiol-conjugates. The literature indicates that some of the oxidized derivatives of CYS-DA that are produced have toxic effects on the brain. However, we found no evidence of CYS-DA in the CSF during intravenous infusion of dopamine for several hours in endotoxemic rats. The extent to which the oxidized derivatives of CYS-DA are toxic outside the brain is not known. One clinical report suggests that

overt toxic effects are not seen in the short term following treatment with high doses of dopamine. Two people were reported to have left hospital neurologically normal after receiving 33 to 25 μg/kg/min dopamine for three to seven hours for treatment of cardiac arrest [25]. We were unable to find any studies that examined the long-term neurological or systemic consequences of dopamine treatment.

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REFERENCES

1. GRAHAM DG, TIFFANY SM, BELL WR JR, GUTNECHT WF: Autoxidation versus covalent binding of quinones as the mechanism of toxicity of dopamine, 6-hydoxydopamine, and related compounds toward C1300 neuroblastoma cells in vitro. *Mol Pharmacol* 14:644–653, 1978
2. MICHEL PP, HEFTI F: Toxicity of 6-hydroxydopamine and dopamine for dopaminergic neurons in culture. *J Neurosci Res* 26:428–435, 1990
3. GIUNTA S, GALEAZZI L, GROPPA G: An in vitro bacterial model of cytotoxicity to living cells caused by dopamine and 6-hydroxydopamine oxidation at physiological pH. *Free Radic Biol Med* 10:297–303, 1991
4. ROSENGREN E, LINDER-ELIASSON E, CARLSSON A: Detection of 5-S-cysteinyldopamine in human brain. *J Neural Trans* 63:247–253, 1985
5. PLOEMEN JH, VAN OMMEN B, DE HAAN A, *et al*: Inhibition of human glutathione S-transferase by dopamine, alpha-methyldopa and their 5-S-glutathionyl conjugates. *Chem Biol Interact* 90:87–99, 1994
6. TSE DCS, MCCREERY RL, ADAMS RN: Potential oxidative pathways of brain catecholamines. *J Med Chem* 19:37–40, 1976
7. SHEN XM, ZHANG F, DRYHURST G: Oxidation of dopamine in the presence of cysteine: Characterization of new toxic products. *Chem Res Toxicol* 10:147–155, 1997
8. SHEN XM, XIA B, WRONA MZ, DRYHURST G: Synthesis, redox properties, in vivo formation, and neurobehavioral effects of N-acetylcysteinyl conjugates of dopamine: Possible metabolites of relevance to Parkinson's disease. *Chem Res Toxicol* 9:1117–1126, 1996
9. MONTINE TJ, PICKLO MJ, AMARNATH V, *et al*: Neurotoxicity of endogenous cysteinylcatechols. *Exp Neurol* 148:26–33, 1997
10. SHEN XM, DRYHURST G: Further insights into the influence of L-cysteine on the oxidation chemistry of dopamine: Reaction pathways of potential relevance to Parkinson's disease. *Chem Res Toxicol* 9:751–763, 1996
11. GOODE HF, WEBSTER NR: Free radicals and antioxidants in sepsis. *Crit Care Med* 21:1770–1776, 1993
12. HAGLUND U, GERDIN B: Oxygen free radicals and circulatory shock. *Circ Shock* 34:405–411, 1991
13. KILGORE KS, LUCCHESI BR: Reperfusion injury after myocardial infarction: The role of free radicals and the inflammatory response. *Clin Biochem* 26:359–370, 1993
14. HUANG Y-L, SALJO A, SUNESON A, HANSSON H-A: A new approach for multiple sampling of cisternal cerebrospinal fluid in rodents with minimal trauma and inflammation. *J Neurosci Methods* 63:13–22, 1995
15. MATTHEWS SB, HENDERSON AH, CAMPBELL AK: The adrenochrome pathway: The major route for adrenaline catabolism by polymorphonuclear leukocytes. *J Mol Cell Cardiol* 17:339–348, 1985
16. ZHANG F, DRYHURST G: Effects of L-cysteine on the oxidation chemistry of dopamine: New reaction pathways of potential relevance to idiopathic Parkinson's disease. *J Med Chem* 37:1084–1098, 1994
17. BURCH PT, AMAN M, BURCH JW: Modification of glutathione content in platelet concentrates by the use of acivicin. *Transfusion* 34:421–426, 1994
18. SHEN XM, DRYHURST G: Oxidation chemistry of (−)-norepinephrine in the presence of L-cysteine. *J Med Chem* 39:2018–2029, 1996
19. SCHEULEN M, WOLLENBERG P, BOLT HM, *et al*: Irreversible binding of DOPA and dopamine metabolites to protein by rat liver microsomes. *Biochem Biophys Res Commun* 66:1396–1400, 1975
20. GABBY M, TAUBER M, PORAT S, SIMANTOV R: Selective role of glutathione in protecting human neuronal cells from dopamine-induced apoptosis. *Neuropharmacology* 35:571–578, 1996
21. MONKS TJ, LAU SS: Toxicology of quinone thioethers. *Crit Rev Toxicol* 22:243–270, 1992
22. BINDOLI A, RIGOBELLO MP, DEEBLE DJ: Biochemical and toxicological properties of the oxidation products of catecholamines. *Free Radic Biol Med* 13:391–405, 1992
23. HASTINGS TG, LEWIS DA, ZIGMOND MJ: Role of oxidation in the neurotoxic effects of intrastriatal dopamine injections. *Proc Natl Acad Sci USA* 93:1956–1961, 1996
24. OFFEN D, ZIV I, STERNIN H, *et al*: Prevention of dopamine-induced cell death by thiol antioxidants: Possible implications for treatment of Parkinson's disease. *Exp Neurol* 141:32–39, 1996
25. OLSEN KH, KLUGER J, FIELDMAN A: Combination high dose amrinone and dopamine in the management of moribund cardiogenic shock after open heart surgery. *Chest* 94:503–506, 1988