3-O-METHYLDLOBUTAMINE, A MAJOR METABOLITE OF DOBUTAMINE IN HUMANS

MAOHE YAN, LESLIE T. WEBSTER, JR., AND JEFFREY L. BLUMER

Departments of Pediatrics and Pharmacology, Case Western Reserve University, Division of Pediatric Pharmacology and Critical Care, Rainbow Babies and Children’s Hospital of the University Hospitals of Cleveland, Cleveland, Ohio

(Received September 25, 2001; accepted January 19, 2002)

This article is available online at http://dmd.aspetjournals.org

ABSTRACT:

Dobutamine is a synthetic ionotropic catecholamine commonly used to treat heart failure and shock. The catabolic fate of dobutamine in humans has yet to be reported, although formation of 3-O-methyldobutamine represents the principal pathway of dobutamine disposition in the dog. Herein, we describe the isolation and identification of 3-O-methyldobutamine in the urine of children receiving infusions of racemic dobutamine. In a 9-year-old child with heart failure ~80% of dobutamine administered intravenously at steady state was detected in the urine. Forty-seven percent of infused dobutamine was identified as 3-O-methyldobutamine and its acid-hydrayed derivatives, the latter mostly conjugated with sulfate (33%). Thirty-two percent consisted of acid-hydrayed dobutamine metabolites, primarily conjugated with sulfate (16%). Sulfate conjugates of both 3-O-methyldobutamine and dobutamine predominated, comprising 33 and 16% of the infused dopamine. In vitro experiments disclosed that crude sonicates of mononuclear cells isolated from human blood catalyzed the formation of 3-O-methyldobutamine from dobutamine and S-adenosylmethionine.

Dobutamine (Fig. 1) is an ionotropic synthetic catecholamine commonly used alone or with other adrenergic agents for temporary support of patients with low cardiac output states and shock (Latifi et al., 2000). Given as a racemic mixture of (+)- and (−)-isomers with differing activity at α- and β-adrenergic receptors, this compound enhances cardiac output primarily by increasing stroke volume with usually only modest chronotropic effects. Dobutamine is given by intravenous infusion because it disappears rapidly from the systemic circulation.

The metabolic disposition of infused dobutamine has yet to be defined in humans. Catecholamines introduced directly into the bloodstream are generally metabolized by catechol-O-methyltransferase (COMT[1] and monoamine oxidase in most animal species, whereas dietary or orally administered catecholamines are commonly conjugated with sulfate (Kopin, 1985; Eisenhofer et al., 1999; Dooley et al., 2000). However, formation of 3-O-methyldobutamine (Fig. 1) via the action of COMT followed by conjugation with glucuronide constitutes the major metabolic route of dobutamine disposition in the dog (Murphy et al., 1976). Whether 3-O-methyldobutamine and/or its conjugates contribute to the overall cardiovascular effects of dobutamine or whether these compounds interact with other drugs in human patients requires elucidation, especially because 3-O-methyldobutamine can act as a potent inhibitor of α1-adrenoreceptors in isolated in vivo systems (Ruffolo et al., 1985).

Herein, we report the isolation of 3-O-methyldobutamine from urine samples of pediatric patients treated with racemic dobutamine. Moreover, about 80% of dobutamine administered intravenously at steady state to a child with heart failure was detected in the urine, largely as 3-O-methyldobutamine-related derivatives (47%) and dobutamine metabolites (32%). Sulfate conjugates of both 3-O-methyldobutamine and dobutamine predominated, comprising 33 and 16% of the infused dopamine. In vitro experiments disclosed that crude sonicates of mononuclear cells isolated from human blood catalyzed the formation of 3-O-methyldobutamine from dobutamine and S-adenosylmethionine.

Materials and Methods

Chemicals. S-Adenosyl-L-methionine (SAM), Tris (hydroxymethyl) aminomethane base, bovine serum albumin, adenosine deaminase (EC 3.5.4.4; 78 U/mg of protein) from bovine spleen, β-glucuronidase (EC 3.2.1.31; 1 × 106 U/mg of protein) from bovine liver, and partially purified sulfatase (EC 3.1.6.1; 2–5 U/mg of protein) from Aerobacter aerogenes were obtained from Sigma Chemical (St. Louis, MO). Sodium octylsulfate was purchased from Eastman Kodak (Rochester, NY), partially purified porcine liver COMT (EC 2.1.1.6, 2200 U/mg of protein) from Calbiochem (La Jolla, CA), and (−)-dobutamine from Sigma/RBI (Natick, MA). HPLC-grade acetone, ethyl acetate, and methylene chloride were obtained from Burdick and Jackson Labs (Muskegon, MI) and acid-washed alumina from Bioanalytical Systems (West Lafayette, IN). Deuterated chloroform was purchased from Merck (Whitehouse Station, NJ). HCl, HClO4, NaH2PO4, and HPLC-grade 85% phosphoric and glacial acetic acids and other reagent-grade chemicals were supplied by Fisher Scientific (Pittsburgh, PA).

Collections from Patients. At this institution urine samples are routinely collected and subjected to various clinical laboratory tests for each patient’s benefit before being discarded. Accordingly, although patient confidentiality was maintained, parental permission was not routinely sought to save urine samples used for the experimental determinations described herein. Urine from several pediatric patients undergoing intravenous therapy with racemic dobut-
amine for cardiac support was saved for isolation and purification of the 3-O-methyldobutamine standard. These collections were pooled and stored at −70°C before further processing. For the partial balance study done in a 9-year-old white male with cardiac failure (Table 3) urine was collected both before (control) and during a timed 5.5-h (experimental) period beginning 48 h after a continuous dobutamine intravenous infusion prescribed at 3 μg min⁻¹ kg⁻¹. A sample of the infusate was stored at −70°C for later determination of dobutamine. Control and experimental urine samples also were stored at −70°C for later analyses of free dobutamine and total dobutamine released by acid hydrolysis, dobutamine sulfate and dobutamine glucuronide, free 3-O-methyldobutamine and total 3-O-methyldobutamine released by acid hydrolysis, 3-O-methyldobutamine sulfate, and 3-O-methyldobutamine glucuronide. The total volume and pH of collected urine were recorded before sample storage.

Blood used for assays of COMT activity in isolated mononuclear cell preparations was obtained from two normal adult volunteers.

**Equipment.** Liquid chromatography with electrochemical detection (HPLC-EC) was carried out with an LC-400 Bioanalytical Systems liquid chromatograph equipped with a carbon/carbon electrode and interfaced with a Varian Instruments (Sunnyvale, CA) model 2510 pump. The potential of the working electrode was maintained at +700 mV versus a Ag⁺/AgCl reference electrode. Separations were achieved in a reversed-phase system with a Bioanalytical Systems phase II ODS stainless steel prepacked column used as the stationary phase (100 μm i.d.; particle size 3 μm). The mobile phase consisted of 880 ml of 0.069 M acetic acid, 2 mM Na₂EDTA adjusted to pH 4.5 with 5 M NaOH before addition of 120 ml of acetonitrile; the resulting mixture was maintained at 70°C before addition of 0.1 ml of sample, the flow rate used for chromatographic separation of 3-O-methyldobutamine was 4.5 ml/min. After manual injection of 0.1 ml of sample, the flow rate used for analysis was adjusted to 0.5 ml/min. The acceleration voltage used was 4000 V, the working electrode was maintained at 700 mV versus a Ag⁺/AgCl reference electrode.

**Preparation of Reference 3-O-Methyldobutamine.** To establish conditions for chromatographic separation of 3-O-methyldobutamine from human urine, the reference metabolite was generated enzymatically from dobutamine, SAM, and partially purified porcine COMT essentially as described by Allen et al. (1992). Reaction mixtures (0.55 ml) contained 0.1 M NaH₂PO₄ buffer, pH 7.4, 0.125 mM dobutamine, 218 μM SAM, 10.9 mM MgCl₂, and 3 U (38 μg of protein) of adenosine deaminase. Reactions were initiated by addition of 25 units of COMT (−10 μg of protein) and proceeded for 45 min at 37°C when they were stopped by addition of 0.5 ml of 1.33 M sodium borate buffer, pH 11, containing 1% (w/v) Na₂EDTA, followed immediately by 5 ml of methylene chloride. After vigorous vortex mixing (30 s) the lower organic phase was removed and evaporated to dryness under vacuum. The residue was reconstituted in 0.2 ml of mobile phase solution, filtered through a nylon microfilter (0.2-μm pore size), and 0.1 ml was injected into the HPLC system for chromatographic analysis.

**Preparation of 3-O-Methyldobutamine Standard from Human Urine.** 3-O-Methyldobutamine used as the external standard was isolated and purified from acid-hydrolyzed pooled samples of urine from dobutamine-treated patients. For acid hydrolysis, 5 ml of thawed urine adjusted to pH 3 with 6 M HCl was mixed with 0.2 ml of 12 M HCl and incubated at 90°C for 30 min after which the mixture was cooled and adjusted to pH 6.5 with 3 M NaOH. Subsequent extraction and processing for reverse phase HPLC followed the same procedure used for enzymatically prepared reference 3-O-methyldobutamine. Fractions eluting with the same retention time as reference 3-O-methyldobutamine were pooled and evaporated to dryness with a Savant Speed Vac concentrator (Farmington, NY). For further purification, residues from many chromatographic separations were taken up in 0.5 ml of mobile phase solution and chromatographed again. Fractions containing a single large peak eluting at the 3-O-methyldobutamine reference retention time were combined, evaporated to dryness, dissolved in 0.5 ml of purified water, extracted into 5 ml of methylene chloride, back extracted with 1 ml of 0.1 N HCl, and vortex mixed. After centrifugation, the upper aqueous layer was removed, evaporated to dryness, taken up in 0.5 ml of water, added to 0.5 ml of 1.33 M sodium borate buffer, pH 11, containing 1% (w/v) Na₂EDTA, extracted into 4 ml of chloroform, and dried in the Savant Speed Vac concentrator. The repurified material was used as the 3-O-methyldobutamine standard.

**Determinations of Free Dobutamine and Urinary Total Dobutamine, Dobutamine Sulfate, and Dobutamine Glucuronide.** Thawed samples of urine and analyzed for free dobutamine were not subjected to acid hydrolysis, whereas urine samples analyzed for total dobutamine (i.e., dobutamine plus its acid-hydrolyzed metabolites) were first subjected to the acid hydrolysis and neutralization steps described above for preparation of the 3-O-methyldobutamine standard from human urine. Before analysis all urinary samples were diluted 10-fold with Millipore water. Then 50 μl of acid-washed alumina was added to 0.2 to 2.0 ml of diluted sample in a 12 × 75-mm polypropylene tube and the mixture was stoppered and vortexed for 3 s. After addition of 1 ml of 1 M Tris·Cl⁻ buffer, pH 8.65, the contents were mixed on a roto-torque (setting 5.5) for 10 min and then centrifuged at 500g for 30 s. The supernatant was discarded and the alumina was washed three times with 2 ml of Millipore water. The slurry with the last wash was transferred to a microfilter tube before centrifugation at 500g for 3 min. The filtered water wash was discarded, a dry receiver tube was substituted, and O.2 ml of 0.1 M HClO₄ was added. This mixture was vortexed for 2 s, allowed to stand for 5 min, vortexed again for 2 s, and centrifuged at 500g for 3 min. Exactly 0.1 ml of the filtered extract containing dobutamine (about 0.2 μl) was injected for the HPLC analysis done as described for 3-O-methyldobutamine. Under these conditions, dobutamine eluted as a single peak at 10 min and recovery of a dobutamine standard was 70%.

For analysis of urinary dobutamine sulfate, 0.05 ml of thawed urine sample was added to 0.45 ml of 0.01 M Tris·Cl⁻ buffer, pH 7.5, and incubated with 2.2 mg of partially purified *Aerobacter aerogenes* sulfatase for 6.5 h at 37°C. The reaction was halted by chilling on ice and the sample was processed and chromatographed as described above for dobutamine. For determination of dobutamine glucuronide, 0.05 ml of a thawed urine sample was added to 0.45 ml of 0.1 M potassium citrate buffer, pH 5.0, and incubated with 5 mg of partially purified bovine β-glucuronidase for 16 h at 37°C. The reaction was stopped by chilling on ice and the pH was adjusted to 6.5 before processing and chromatography as described above for dobutamine.

**Determinations of Free and Total 3-O-Methyldobutamine, 3-O-Methyldobutamine Sulfate, and 3-O-Methyldobutamine Glucuronide in Urine.** To determine total 3-O-methyldobutamine in the urine, both control (before dobutamine therapy) and experimental (during dobutamine therapy) urine samples were thawed, subjected to acid hydrolysis, and chromatographed as described for the purified urinary 3-O-methyldobutamine standard. Processing of samples used to quantitate free 3-O-methyldobutamine in the urine was identical except that the acid hydrolysis step was omitted. For 3-O-methyldobutamine sulfate analysis, 0.05 ml of a thawed urine sample was added to 0.45 ml of 0.01 M Tris·Cl⁻ buffer, pH 7.5, and incubated with 2.2 mg of partially purified *Aerobacter aerogenes* sulfatase for 6.5 h at 37°C. The reaction was stopped by chilling on ice and the sample was extracted and chromatographed as described for 3-O-methyldobutamine. For determination of 3-O-methyldobutamine glucuronide, 0.05 ml of a thawed urine sample was added to 0.45 ml of 0.1 M potassium citrate buffer, pH 5.0, and incubated with 5 mg of partially purified bovine β-glucuronidase for 16 h at 37°C. The reaction was stopped by chilling on ice and the pH was adjusted to 6.5 before processing and chromatography as described above for dobutamine.

**Fig. 1. Chemical structures of dobutamine (A) and 3-O-methyldobutamine (B).**
TABLE 1

Recoveries of dobutamine, dopamine, 3-O-methyldobutamine, and 3-methoxytyramine from porcine COMT assay mixtures after extraction with methylene chloride or ethyl acetate

<table>
<thead>
<tr>
<th></th>
<th>Methylene Chloride</th>
<th>Ethyl Acetate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± S.D. %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dobutamine</td>
<td>0.3 ± 0.05</td>
<td>11.4 ± 2.5</td>
</tr>
<tr>
<td>Dopamine</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>3-O-Methyldobutamine</td>
<td>36.7 ± 3.5</td>
<td>36.3 ± 5.1</td>
</tr>
<tr>
<td>3-Methoxytyramine</td>
<td>42.7 ± 5.4</td>
<td>40.3 ± 4.0</td>
</tr>
</tbody>
</table>

dobutamine glucuronide, 0.05 ml of a thawed urine sample was added to 0.45 ml of 0.1 M potassium citrate buffer, pH 5.0, and incubated at 37°C with 5 mg of partially purified bovine β-glucuronidase for 16 h. The reaction was terminated by chilling on ice, and the pH of the sample was adjusted to 6.5 before extraction and chromatographic analysis as outlined for 3-O-methyldobutamine.

Assays for Catechol-O-Methyltransferase Activity in Human Blood Mononuclear Cells. Formation of 3-O-methyldobutamine from dobutamine and SAM was assayed as described above for enzymatic formation of reference 3-O-methyldobutamine except that human mononuclear cell sonicates (0.30–0.44 mg of protein/0.55-ml reaction mixture) were substituted for porcine COMT. Human mononuclear cells from 3 ml of blood were separated, washed, sonicated, and stored at −70°C as described by Allen et al. (1992); protein concentrations in the sonicates were determined by the method of Lowry et al. (1951), with bovine serum albumin used as the standard. Reactions were run for 45 min after which methods for terminating the reaction, and extracting and chromatographing the product were identical to those used to generate the reference 3-O-methyldobutamine with porcine enzyme. Recovery and quantitation of the 3-O-methyldobutamine product was achieved by spiking control and experimental reaction mixtures with known amounts of 3-O-methyldobutamine standard repurified from urine of dobutamine-treated patients.

Results

3-O-Methyldobutamine generated enzymatically from dobutamine with partially purified porcine COMT served as the reference used to establish optimal conditions for the extraction and chromatographic separation of this metabolite from urine. Conditions for 3-O-methyldobutamine generation were those used by Allen et al. (1992), to assay human blood mononuclear cell COMT activity with dopamine as the substrate. However, ethyl acetate, the organic solvent used to extract the dopamine reaction products, coextracted over 10% of the dobutamine substrate, which interfered with the subsequent HPLC product analysis (Table 1). Substitution of methylene chloride for ethyl acetate selectively reduced the amount of coextracted dobutamine. The latter was detected as a large peak in fractions eluting at 21 min that depended on the presence of dobutamine, SAM, and active porcine enzyme in the original reaction mixture (Fig. 2, peak B). Mass spectral analysis of material in the 21-min fraction revealed a molecular ion signal at 315 m/z, consistent with its identity as 3-O-methyldobutamine (data not shown). The substance eluting in a minor peak at 30 min (Fig. 2, peak C) was not identified but may have been 4-O-methyldobutamine (see Discussion).

Chromatographic conditions that separated the enzymatically formed reference 3-O-methyldobutamine from dobutamine were used to isolate and purify this metabolite from acid-hydrolyzed urine samples obtained from pediatric patients treated with dobutamine. Repurified material eluting at 21 min from multiple chromatographic separations (Fig. 3) was subjected to both mass spectral and proton NMR analysis (see Materials and Methods).

The largest ion (M+) in the mass spectrum of the repurified material dissolved in methanol was noted at 315.18304, which corresponds closely to the calculated molecular weight of 3-O-methyldobutamine (315.1834) (Fig. 4). The mass spectrum also exhibited several characteristic ion fragments at m/z 107, 137, 178, and 194, which, taken together, are consistent with the identity of the purified material as 3-O-methyldobutamine.

Figure 5 shows the proton NMR spectrum of the repurified urinary metabolite, and Table 2 lists the chemical shifts of the proton signals and their proposed assignments relative to CDCl₃ at 7.3 ppm. Signals of protons on the two aromatic rings were noted at 6.61 to 6.92 ppm, whereas those due to the three protons of the hydroxymethyl group occurred at 3.90 ppm. Signals from most of the remaining saturated hydrocarbon protons were seen at 1.08 to 2.98 ppm, although a few
could not be detected because of resonance broadening and/or overlap. The proton NMR spectrum also is consistent with the identity of the repurified urinary material as 3-O-methyldobutamine.

The repurified urinary metabolite was used as an external standard for extraction, isolation, and quantitation of 3-O-methyldobutamine both in urine and enzymatic reaction mixtures. The extinction coefficient, $\Sigma_{\text{i}}^\text{cm} \text{mM}^{-1} \text{cm}^{-1}$, of this standard dissolved in water was 5.73 optical density units and its recovery from urine was about 36% (Table 1). The sensitivity of the method was 22 ± 5 ng/ml and the between day variation for determinations on different frozen aliquots of the same urine sample was 11.8%.

Methodology devised to measure dobutamine and 3-O-methyldobutamine in addition to their total acid-hydrolyzed derivatives and their sulfate and glucuronide conjugates permitted a partial balance of the urinary dobutamine and 3-O-methyldobutamine metabolites.

Assumptions: 1) and the continuously monitored volume of dobutamine delivered for extraction, isolation, and quantitation of 3-O-methyldobutamine metabolite elimination in urine of a pediatric patient. 5

Comparison of rate of dobutamine infusion at steady state with rates of dobutamine and 3-O-methyldobutamine metabolite elimination in urine of a pediatric patient

**TABLE 2**

<table>
<thead>
<tr>
<th>Proton Designation</th>
<th>No. of Protons</th>
<th>Chemical Shifts</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>3</td>
<td>1.08–1.18</td>
</tr>
<tr>
<td>b</td>
<td>2</td>
<td>1.57–1.88</td>
</tr>
<tr>
<td>c</td>
<td>2</td>
<td>2.11–2.25</td>
</tr>
<tr>
<td>d</td>
<td>2</td>
<td>2.41–2.60</td>
</tr>
<tr>
<td>e</td>
<td>2</td>
<td>2.65–2.80</td>
</tr>
<tr>
<td>f</td>
<td>3</td>
<td>3.90</td>
</tr>
<tr>
<td>g</td>
<td>3</td>
<td>6.61–6.70</td>
</tr>
<tr>
<td>h</td>
<td>2</td>
<td>6.75–6.80</td>
</tr>
<tr>
<td>i</td>
<td>2</td>
<td>6.85–6.92</td>
</tr>
</tbody>
</table>

In vitro experiments revealed that crude preparations of human blood mononuclear cells catalyzed the formation of 3-O-methyldobutamine from dobutamine and SAM. Detection of a product peak eluting at 21 min upon HPLC of the reaction mixture depended on the presence of dobutamine, SAM, and active enzyme in the original reaction mixture. Moreover, a molecular ion signal at 315 m/z was consistent with the presence of 3-O-methyldobutamine in the fraction.

**TABLE 3**

<table>
<thead>
<tr>
<th>Component</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dobutamine (measured)</td>
<td>96</td>
</tr>
<tr>
<td>Dobutamine (prescribed)</td>
<td>100</td>
</tr>
<tr>
<td>Urinary excretion rates</td>
<td></td>
</tr>
<tr>
<td>Dobutamine</td>
<td>1</td>
</tr>
<tr>
<td>Dobutamine sulfate</td>
<td>16</td>
</tr>
<tr>
<td>Dobutamine glucuronide</td>
<td>6</td>
</tr>
<tr>
<td>Dobutamine + conjugates$^a$</td>
<td>33</td>
</tr>
<tr>
<td>3-O-Methyldobutamine</td>
<td>2</td>
</tr>
<tr>
<td>3-O-Methyldobutamine sulfate</td>
<td>33</td>
</tr>
<tr>
<td>3-O-Methyldobutamine glucuronide</td>
<td>3</td>
</tr>
<tr>
<td>3-O-Methyldobutamine $\pm$ its conjugates$^b$</td>
<td>47</td>
</tr>
<tr>
<td>Sum of dobutamine $+$ conjugates$^a$ and 3-O-methyldobutamine $+$ conjugates$^a$</td>
<td>80</td>
</tr>
</tbody>
</table>

$^a$Indicated percentages are relative to the measured dobutamine infusion rate set at 100.

$^b$The measured infusion rate is the product of the analyzed concentration of dobutamine in the infusate (2.078 mg ml$^{-1}$) and the continuously monitored volume of dobutamine delivered over time (15.4 ml over 300 min). The infusion pump was calibrated to an accuracy of ±5%. Data are converted to nanomoles minute$^{-1}$.

$^c$Determined by HPLC as dobutamine after acid hydrolysis of urine.

$^d$Determined by HPLC as 3-O-methyldobutamine after acid hydrolysis of urine.
eluting at 21 min (data not shown). Conditions found optimal for
assaying this crude blood mononuclear cell enzyme with dobutamine
were similar to those described previously for dopamine (see Mate-
rials and Methods; Allen et al., 1992). Thus, at pH 7.4 and 37°C with
0.125 mM dobutamine, 218 μM SAM, 10.9 mM MgCl₂, and 3 units
of adenosine deaminase in 0.55 ml of reaction mixture, formation of
3-O-methyldobutamine was linear with added mononuclear cell pro-
tein concentration and with time up to 60 min.

Discussion

To our knowledge this is the first time that 3-O-methyldobutamine
has been identified as a major metabolite of infused dobutamine in
humans. This compound was isolated from the urine of dobutamine-
treated pediatric patients and identified by its chromatographic behav-
ior and spectral properties. 3-O-Methyldobutamine and its derivatives
also comprised most of the drug forms detected in the urine of a
patient infused with dobutamine at steady state. Moreover, COMT
activity in crude preparations of human blood mononuclear cells was
shown to catalyze the formation of 3-O-methyldobutamine from do-
butamine in vitro.

Two lines of evidence suggest that our isolated urinary dobutamine
metabolite is 3-O-methyldobutamine rather than 4-O-methyldobu-
tamine, even though the latter was not available for direct comparison.
First is that COMT catalyzes the transfer of the methyl group from
SAM predominantly to position 3 of dihydroxyphenyl derivatives
(Kopin, 1985; Lotta et al., 1995; Männisto and Kaakkola, 1999).
Thus, porcine COMT generated a major dobutamine metabolite (Fig.
2, peak B) that eluted before a small peak due to a minor unidentified
dobutamine metabolite, possibly 4-O-methyldobutamine (Fig. 2, peak C).
Material in the major peak fraction had the same retention time (21
min) and molecular ion (m/z 315) as our purified urinary metabolite
standard that exhibited both mass and proton NMR spectra consistent
with the 3-O-methyldobutamine structure. The second observation
supporting the 3-O-methyl structure for our dobutamine metabolite is
that 3-methoxytyramine, a major metabolite of dopamine, elutes be-
fore 4-methoxydopamine under similar chromatographic conditions
(Allen et al., 1992). Tandem mass spectrometry of our standard
urinary dobutamine metabolite could unequivocally establish the po-

tion of its O-methyl group by further fragmentation of the 137 m/z
ion (Fig. 4).

That 47% of dobutamine infused at steady state appeared as 3-O-
methyldobutamine in acid-hydrolyzed urine of our patient (Table 3)
and that appreciable quantities of this metabolite were isolated from
the urine of other pediatric patients treated with dobutamine argue that
3-O-methyldobutamine is a major metabolite of infused dobutamine
in humans. COMT with its preference for catechol substrates and wide
tissue distribution presumably accounts for this finding but the phe-
notype/genotype of this enzyme was not determined in our patient. An
enzyme that exists in both soluble and membrane-bound forms en-
coded by a single gene, COMT exhibits a genetically balanced poly-
morphism with a trimodal distribution of low, intermediate, and high
activities in human tissues (ratio 1:2:1) (Männisto and Kaakkola,
1999; Weinsilboum et al., 1999). Low activity is due to a thermo-
labile form of the enzyme with a single G → A transition that results
in a change from valine to methionine at codon 108 in soluble COMT
and at codon 158 in membrane-bound COMT. Future population
studies should reveal whether different COMT genotypes account for
large differences in the elimination of 3-O-methyldobutamine and its
metabolites in the urine of dobutamine-treated patients.

Most of the dobutamine infused into our patient at steady state was
detected in the urine as unidentified sulfate conjugates of either
3-O-methyldobutamine (33%) or dobutamine (16%) (Table 3). Be-

Because both sulfation and glucuronidation were established indirectly
by enzymatic hydrolysis in the present study, the sites of sulfation and

glucuronidation on 3-O-methyldobutamine and dobutamine await di-
rect isolation and characterization of the individual metabolites [i.e.,
theoretically, conjugation could take place on the catechol ring, the
monophenolic ring, or both (Fig. 1)]. Nonetheless, the unique human
catecholamine sulfotransferase SULT 1A3 is an especially attractive
candidate to explain most of the sulfation observed herein because of
all the human SULTs, this enzyme has the most marked affinity for
catecholamine substrates (e.g., dopamine Km ≈ 1 μM) and a wide
tissue distribution. One of many human cytosolic sulfotransferases that
use phosphoadenosine phosphosulfate as the activated sulfate
donor, SULT 1A3 has been cloned, sequenced, expressed, and char-
acterized in various systems and crystallized at a resolution of 2.4 A
(Brix et al., 1999; Bidwell et al., 1999; Dajani et al., 1999; Dooley et
al., 2000). This thermolabile sulfotransferase is highly expressed in
intestine and because its postprandial activity increases dramatically it
has been suggested to serve as a “gut-blood” barrier for detoxifying
dietary biogenic amines (Eisenhofer et al., 1999; Dooley et al., 2000).
However, much still needs to be learned about the number and
genetics of the human sulfotransferases and the presence of various
sulfotransferases makes the physiological role of particular sulfotransferases
difficult to elucidate (Dooley et al., 2000; Iida et al., 2001).

In the child treated with dobutamine at steady state our partial
balance study accounted for about 80% of the infused drug in the
urine but left the remaining 20% unexplained (Table 3). Unidentified
urinary metabolites of dobutamine might have contributed to this
discrepancy because the urinary analysis was limited to quantifying
dobutamine, 3-O-methyldobutamine, and their acid-hydrolyzed deriv-
atives. But dobutamine also might undergo a significant enterohepatic
circulation due to its chemical structure as a weak organic base and
partial biliary elimination with or without conjugation/deconjugation
recycling. Indeed, dogs infused with C¹⁴-labeled dobutamine excreted
20% of the radiolabel in the stool compared with 67% in the urine and
animals with cannulated bile ducts eliminated 30 to 35% of a radio-
labeled dose in the bile (Murphy et al., 1976). An enterohepatic
circulation of dobutamine in humans might be detected by analyzing
stool samples from dobutamine-treated patients or by testing bile from
dobutamine-treated patients with biliary fistulas for dobutamine and
its metabolites.

That crude sonicates of human blood mononuclear cells could
substitute for porcine COMT in catalyzing the methylation of dobut-
amine in vitro is consistent with previous work that defined optimal
conditions for this preparation with dopamine as the catecholamine
substrate (Allen et al., 1992, 1997). Those studies also provided
evidence that COMT activity in human blood mononuclear cell pre-
parations reflects that found in human red blood cells, which in turn

have been used extensively to characterize the genetics of human
COMT (Weinsilboum et al., 1999). Recent kinetic studies with the
human mononuclear cell preparation reveal that dobutamine and
dopamine act as competitive inhibitors of each other for COMT
activity and that dobutamine serves as the better substrate, largely due
to its lower apparent Km (0.05 versus 0.44 mM) (Yan et al., 2002).
These observations are in accord with a recent structure-activity
kinetic study that showed that both catecholamines serve as substrates
for a human recombinant COMT enzyme (Lautala et al., 2001).

In summary, we present evidence, both in vivo and in vitro, that
formation of 3-O-methyldobutamine is a major pathway of dobut-
amine metabolism in humans. In a pediatric patient treated intrave-
nously with dobutamine at steady state, about 47% of infused dobut-
amine was found in the urine as 3-O-methyldobutamine and its
derivatives. About 49% of the infused drug consisted of urinary
sulfate conjugates of 3-\(O\)-methyldobutamine (33\%) and dobutamine (16\%).

Acknowledgments. We thank Anita Pettigrew and Dr. Carolyn Myers for assistance in developing the analytical methodology and Drs. Carolyn Myers, Wesley A. Gray, and John J. Mieyal for advice and suggestions about the data.

References