Enantiomer Analysis of A New Street Drug, 3,4-Methylenedioxy-N-methyl-butanamine, in Rat Urine

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Abstract

A new street drug, 3,4-methylenedioxy-N-methyl-butanamine (MBDB), has been found in Japan recently. The stereoisomer monitoring and the urinary excretion kinetics are not determined in biological fluids even though abused MBDB is a racemic form [enantiomer ratio (-/+)=1.00]. The present studies were done by high-performance liquid chromatography (HPLC) equipped with a chiral activity column at 40°C using urine specimens from five Wistar rats. Urine samples were collected over six time intervals after a single oral administration of racemic MBDB (30 mg/kg).

Unchanged MBDB and 3,4-methylenedioxybutanamine (BDB), an N-demethylated metabolite, were found in the rats’ urine. Each enantiomer of MBDB and BDB was monitored (peak resolution > 1.00) by HPLC analysis within 30 min. For both MBDB and BDB, the (+)-isomers were excreted a little more than the (-)-isomers. The stereoselective disposition of BDB was more remarkable than that of MBDB and was observed in the urine throughout the study (p < 0.05). The urinary excretion of MBDB showed significant difference between the two enantiomers from 4 to 24 h (p < 0.05). The amount of MBDB excreted up to 24 h was 34.7 ± 2.8% of the administered dose: 17.6 ± 1.4% for (+)-isomer and 17.1 ± 1.5% for (-)-isomer. The amount of BDB was 4.9 ± 1.0%; 2.9 ± 0.6% for (+)-isomer and 2.0 ± 0.4% for (-)-isomer. The enantiomer ratio (-/+ ) of MBDB and BDB was 1.00 or a little smaller. The ratio (-/+ ) of MBDB changed from 1.00 ± 0.02 to 0.88 ± 0.09 by 24 h, and that of BDB from 0.68 ± 0.03 to 0.78 ± 0.02. The ratio (-/+ ) for MBDB and BDB accumulated up to 24 h was 0.97 ± 0.01 and 0.70 ± 0.06, respectively, and the total ratio (-/+ ) of the two substances was 0.93 ± 0.02 (p < 0.05). These findings suggested that the stereoselective disposition of racemic MBDB was different from that of 3,4-dimethylenedioxyamphetamine and 3,4-dimethylenedioxyamphetamine, and was similar to that of methamphetamine. In addition, the enantiomer ratio of MBDB and BDB has not been reported as yet, but MBDB tablets have been confiscated from methamphetamine users recently, and its abuse has become a threat as a new class of street drug. The regulatory system against its production has been promulgated, but among hallucinogenic drugs only MBDB is not yet legally restricted in Japan. On the other hand, 3,4-methylenedioxyamphetamine (MDA) analogues, which have similar structures to MBDB, are known as drugs of high abuse like cannabis and cocaine in the U.S. and European countries. They are among the drugs that are often handled in the practical fields of forensic chemistry and forensic toxicology in each country. Furthermore, death caused by an overdose has also been reported (12). These MDA analogues are strictly regulated in Japan like cocaine and morphine. The chemical structure of MBDB is similar to that of MDA analogues, and the absolute configurations of the R(−) and S(+) enantiomers of MBDB are also the same as those of MDA analogues. MBDB tablets confiscated in Japan were of a racemic form. It is likely that in countries other than Japan this racemic type is being abused. The detailed metabolic fate of MBDB has not been studied in either humans or animals. Metabolic products such as the N-demethylated compound, 3,4-methylenedioxybutanamine (BDB), and the methylated dihydroxy compound, N-methyl-1-(4-hydroxy-3-thoxyphenyl)-butanamine, of MBDB origin have been found in the urine of MBDB abusers (7,17,13). These metabolites are simultaneously analyzed in the form of the racemic compound, and the enantiomer kinetics have not been studied in biological fluids. Furthermore, identification of the optically active compounds and kinetic studies on racemic MBDB has not been done yet.

Introduction

3,4-Methylenedioxy-N-methyl-butanamine (MBDB), which is the α-ethyl homologue of 3,4-methylenedioxyamphetamine (MDMA), has an MDMA-like stimulant action and is distinctly different from the hallucinogenic 3,4-methylenedioxyamphetamine (1-6). Furthermore, this drug is a substance belonging to the new drug class of “entactogens” (1,2). According to the Kintz report (7), MBDB is now listed as a controlled substance in the United States and France as a highly addictive drug because of its unique pharmacological properties. Its abuse has been noted in countries such as Spain, Sweden, Italy, Germany, the U.S., and France according to the analytical literature (8-11). In Japan, the abuse of this drug has not been reported as yet, but MBDB tablets have been confiscated from methamphetamine users recently, and its abuse has become a threat as a new class of street drug. The regulatory system against its production has been promulgated, but among hallucinogenic drugs only MBDB is not yet legally restricted in Japan. On the other hand, 3,4-methylenedioxyamphetamine (MDA) analogues, which have similar structures to MBDB, are known as drugs of high abuse like cannabis and cocaine in the U.S. and European countries. They are among the drugs that are often handled in the practical fields of forensic chemistry and forensic toxicology in each country. Furthermore, death caused by an overdose has also been reported (12). These MDA analogues are strictly regulated in Japan like cocaine and morphine. The chemical structure of MBDB is similar to that of MDA analogues, and the absolute configurations of the R(−) and S(+) enantiomers of MBDB are also the same as those of MDA analogues. MBDB tablets confiscated in Japan were of a racemic form. It is likely that in countries other than Japan this racemic type is being abused. The detailed metabolic fate of MBDB has not been studied in either humans or animals. Metabolic products such as the N-demethylated compound, 3,4-methylenedioxybutanamine (BDB), and the methylated dihydroxy compound, N-methyl-1-(4-hydroxy-3-thoxyphenyl)-butanamine, of MBDB origin have been found in the urine of MBDB abusers (7,11,13). These metabolites are simultaneously analyzed in the form of the racemic compound, and the enantiomer kinetics have not been studied in biological fluids. Furthermore, identification of the optically active compounds and kinetic studies on racemic MBDB has not been done yet.
Therefore, we thought it of importance to separate each isomer in biological fluids in order to do the kinetic study. Our present study deals with the simultaneous monitoring of the two optical isomers of non-hallucinogen MBDB and their excretion kinetics in rat urine.

Materials and Methods

Synthesis and reagents
The racemates of MBDB and BDB were synthesized by the method of Nichols et al. (1) and Noggle et al. (14).
Levorotatory (−)-methamphetamine used as the internal standard was obtained from Dr. Tatsuo Nagai (Department of Forensic Science, School of Allied Health Science, Kitasato University, Kanagawa, Japan). Normal-hexane, 2-propanol, and benzoyl chloride were obtained from Wako Pure Chemical (Osaka, Japan). All other chemicals were purchased from commercial sources.

Animal and urine samples
Five male Wistar rats (6 weeks old, 215 ± 5 g) were purchased from Charles River Japan, Inc. (Tsukuba, Japan). Racemic MBDB hydrochloride was dissolved in saline, and a dose of 30-mg/kg-body weight was administered orally to rats through a stomach tube. Rat urine specimens were collected over six intervals, 0-4, 4-8, 8-12, 12-16, 16-20, and 20-24 h, after administration of racemic MBDB. Urinary pH varied from 6.2 to 6.8.

Purity and optical activity tests
The purity of two synthetic psychoactive agents, MBDB and BDB, was found to be more than 98% when analyzed by gas chromatography and gas chromatography–mass spectrometry. The optically active forms (Figure 1) of MBDB and BDB were identified with a polarimeter. Determination of the enantiomer ratio (−/+) was done by high-performance liquid chromatography (HPLC) equipped with a chiral column. The MBDB and BDB were shown be a racemate (50% (+)-isomer and 50% (−)-isomer). The ratios (−/+) for both MBDB and BDB were 1.01 ± 0.01 (n = 5).

Extraction and quantitation
Rat urine (0.1 to 0.3 mL) was mixed with 0.1 mL of 1.5M sodium hydroxide and 2.5 μg of an internal standard (l-(−)-methamphetamine). The final volume of urine was adjusted to 1.0 mL with distilled water. The urine was then transferred to an Extrelut column (Merck). The enantiomers of MBDB and BDB were eluted from the column 15 min later with 6.0 mL of n-hexane, followed by back extraction with 1.0 mL of 0.1M sulfuric acid. The sulfuric acid layer, together with 3.0 mL of 2.5M sodium hydroxide and 10.0 μL of benzoyl chloride, was then stirred vigorously with a mixer. Benzoyl derivatives of MBDB and BDB were re-extracted with 3.0 mL of n-hexane, washed twice with 2.0 mL of distilled water, and dried in a water bath at 40°C with N₂ gas. The residue was dissolved in 200 μL of n-hexane/2-propanol (87:13, v/v), and 20-μL aliquots were injected for HPLC analysis. Recovery of MBDB and BDB isomers was estimated after adding 10 μg of each isomer to drug-free rat urine (0.2 mL). Quantitation was performed with a calibration curve based on the peak-area ratio of each isomer to the peak area of l-methamphetamine (internal standard) and were done in the concentration range within the calibration range. The regression line in the 0–15.0-μg/mL concentration range was \( y = 0.003x + 0.060 \) (n = 5, r = 0.995) for MBDB and \( y = 0.003x + 0.110 \) (n = 5, r = 0.997) for BDB.

Table I. Comparison of the Ability of HPLC to Separate Racemic MBDB and BDB in Rat Urine Measured with Two Different Analysis Columns*

<table>
<thead>
<tr>
<th>Column condition</th>
<th>Drug</th>
<th>Peak resolution (Rs)</th>
<th>Retention time (min)</th>
<th>Recovery (%)</th>
<th>Enantiomer ratio (−/+)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(+) form</td>
<td>(-) form</td>
<td>(+) form</td>
<td>(-) form</td>
</tr>
<tr>
<td>A*</td>
<td>MBDB</td>
<td>2.64</td>
<td>11.0</td>
<td>14.2</td>
<td>76.5</td>
</tr>
<tr>
<td></td>
<td>BDB</td>
<td>1.68</td>
<td>14.7</td>
<td>16.9</td>
<td>73.5</td>
</tr>
<tr>
<td>B*</td>
<td>MBDB</td>
<td>1.68 ± 0.01</td>
<td>18.3 ± 0.03</td>
<td>21.7 ± 0.03</td>
<td>75.5 ± 3.4</td>
</tr>
<tr>
<td></td>
<td>BDB</td>
<td>1.17 ± 0.01</td>
<td>24.7 ± 0.03</td>
<td>27.2 ± 0.03</td>
<td>71.1 ± 3.6</td>
</tr>
</tbody>
</table>

* Columns are a single Chiralcel OJ column (A) and two Chiralcel OJ connection columns (B).  
* A. Value is the result of a single determination.  
* B. Each value represents an average of five determinations; values are the mean plus or minus standard deviation.
column (25 cm x 4.5-mm i.d., Daicel Chemical Ind., Tokyo, Japan). The detectors used were an UV-8000 ultraviolet spectrophotometer (TOSO Co., Tokyo, Japan) and a Shodex OR-2™ polarimeter (Showa Electric, Tokyo, Japan). The detection wavelength was set at 220 nm for the ultraviolet (UV) detector and 450 nm for the optical rotation (OR) detector. The UV wavelength corresponds to the maximum absorption of the benzoyl derivatives of MBDB and BDB, and the OR wavelength is fixed at 450 nm because of the mercury lamp. The column temperature was maintained at 40°C. The mobile phase was n-hexane/2-propanol (87:13, v/v), and the flow rate was 1.0 mL/min.

**Results**

Analytical accuracy of MBDB and BDB enantiomers

The analytical accuracy of authentic racemates, MBDB and BDB, in the rat urine was estimated from peak resolution (Rs), retention time (Rt) and the enantiomer ratio (+/-) as shown in Table I. With a single OJ column, the simultaneous analytical time of two authentic substances was within 20 min, and the (-)-isomer of MBDB and the (+)-isomer of BDB overlapped. The separation was not improved by changing the column temperature or the flow rate. The ratios (+/-) were 0.72 for racemic MBDB and 0.86 for racemic BDB, and they did not correspond to the theoretical values of 1.00 for the racemate of each of the two authentic substances. From these results, the isomer analysis by two OJ connection columns was then attempted (Table I). In this method, the simultaneous analysis time of MBDB and BDB isomers was approximately 30 min, and the four isomers were completely separated. For MBDB and BDB, the Rs were 1.68 ± 0.01 (n = 5) for MBDB and 1.17 ± 0.01 (n = 5) for BDB. The ratio (+/-) was 1.01 ± 0.02 (n = 5) for racemic MBDB and 1.01 ± 0.01 (n = 5) for racemic BDB, and almost equaled to the theoretical value of 1.00 for the authentic racemates of MBDB.

**Table II. Excretion Kinetic of (+)- and (-)-Enantiomers of Unchanged MBDB and Its N-Demethylated Substance, BDB, in Rat Urine Specimens after a Single Oral Administration of Racemic MBDB**

<table>
<thead>
<tr>
<th>Collection time (h)</th>
<th>(+)-MBDB (%)</th>
<th>(-)-MBDB (%)</th>
<th>Total (%)</th>
<th>(+)-BDB (%)</th>
<th>(-)-BDB (%)</th>
<th>Total (%)</th>
<th>Ratio</th>
<th>(+) form (%)</th>
<th>(-) form (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-4</td>
<td>4.79 ± 0.79</td>
<td>4.79 ± 0.79</td>
<td>9.58 ± 0.30</td>
<td>0.20 ± 0.30</td>
<td>0.51 ± 0.51</td>
<td>18.87 ± 1.10</td>
<td>5.09 ± 0.84</td>
<td>0.20 ± 0.03</td>
<td>10.89 ± 0.72</td>
<td></td>
</tr>
<tr>
<td>4-8</td>
<td>4.70 ± 0.74</td>
<td>4.61 ± 0.64</td>
<td>9.32 ± 0.64</td>
<td>0.45 ± 0.64</td>
<td>1.00 ± 1.00</td>
<td>6.98 ± 1.06</td>
<td>5.35 ± 0.84</td>
<td>0.10 ± 0.03</td>
<td>10.41 ± 0.73</td>
<td></td>
</tr>
<tr>
<td>8-12</td>
<td>4.63 ± 0.70</td>
<td>4.46 ± 0.60</td>
<td>9.10 ± 0.60</td>
<td>0.65 ± 0.65</td>
<td>1.65 ± 1.65</td>
<td>4.85 ± 1.65</td>
<td>5.64 ± 0.84</td>
<td>0.15 ± 0.03</td>
<td>10.73 ± 0.75</td>
<td></td>
</tr>
<tr>
<td>12-16</td>
<td>1.98 ± 0.76</td>
<td>1.85 ± 1.55</td>
<td>3.84 ± 1.55</td>
<td>0.62 ± 1.62</td>
<td>1.09 ± 1.09</td>
<td>3.90 ± 1.09</td>
<td>2.33 ± 1.65</td>
<td>0.08 ± 0.03</td>
<td>6.11 ± 0.76</td>
<td></td>
</tr>
<tr>
<td>16-20</td>
<td>1.23 ± 1.17</td>
<td>1.24 ± 1.49</td>
<td>2.47 ± 1.49</td>
<td>0.48 ± 1.48</td>
<td>1.07 ± 1.07</td>
<td>4.95 ± 1.07</td>
<td>2.06 ± 1.65</td>
<td>0.06 ± 0.03</td>
<td>4.96 ± 0.76</td>
<td></td>
</tr>
<tr>
<td>20-24</td>
<td>1.36 ± 0.76</td>
<td>0.57 ± 0.70</td>
<td>1.20 ± 0.70</td>
<td>0.11 ± 0.11</td>
<td>0.08 ± 0.08</td>
<td>1.77 ± 0.08</td>
<td>0.74 ± 0.08</td>
<td>0.13 ± 0.03</td>
<td>2.65 ± 0.76</td>
<td></td>
</tr>
<tr>
<td>Total**</td>
<td>1.37 ± 1.45</td>
<td>1.70 ± 1.80</td>
<td>34.71 ± 2.90</td>
<td>2.03 ± 2.03</td>
<td>4.93 ± 4.93</td>
<td>7.28 ± 7.28</td>
<td>20.52 ± 20.52</td>
<td>19.11 ± 19.11</td>
<td>39.63 ± 39.63</td>
<td></td>
</tr>
</tbody>
</table>

* Administered dosage is 30 mg per kg of body weight and corresponds to 6.5 ± 0.2 mg (n = 5).
† Values are the average ratios of the MBDB to the BDB excreted over each collection time.
‡ Each value represents an average of the percentage of administered MBDB made up of the excreted enantiomer; the value is the mean plus or minus standard deviation for five rats.
§ Percentages are the sum of the (+) form and the (-) form.
* Values are the sum of the (+) form and the sum of the (-) form for the unchanged MBDB and its N-demethylated substance, BDB.
** Not significant versus the (+) form (Student's t-test).
*** p < 0.05 versus the (+) form (Student's t-test).
†† Total percentage is the sum of the (+) form and the sum of the (-) form of the metabolites excreted over 24 h.
and BDB. The coefficient of variation was within ± 1.0%. The yield of MBDB and BDB isomers from the rat urine was nearly the same for each isomer as shown in Table I. The coefficient of variation was within ± 5.1%. The ability of HPLC separation for MBDB and BDB isomers by two OJ connection columns was better than that of a single OJ column. On the basis of these results, the two OJ connection columns were used for the identification of MBDB and BDB isomers in rat urine thereafter.

Enantiomer identification and simultaneous analysis

The optical activity identification of authentic racemates, MBDB and BDB, measured with an OR detector is shown in Figure 2A. The (+)-isomer and the (−)-isomer of MBDB and BDB were detected by a mutually opposite optical rotation as shown in Figure 2A. Each isomer on the chromatogram corresponds to 250 μg as an absolute amount. The analysis time was approximately 30 min, and the detection limit was approximately 50 μg per 20 μL injections for each isomer (signal-to-noise ratio = 3). Figure 2B shows a typical HPLC chromatogram of the standard substances, racemic MBDB and BDB. Each isomer applied was 50 ng as the absolute amount. The ratios (−/+) were 1.01 for MBDB and 0.99 for BDB. The detection limit with an UV detector was 2500 times as high as that of the OR detector and was approximately 20 ng per 20μL injections for each isomer (signal-to-noise ratio = 3). Figure 2B shows a typical HPLC chromatogram of the standard substances, racemic MBDB and BDB. Each isomer applied was 50 ng as the absolute amount. The ratios (−/+) were 1.01 for MBDB and 0.99 for BDB. The detection limit with a UV detector was 2500 times as high as that of the OR detector and was approximately 20 ng per 20 μL injections, and the signal-to-noise ratio was 10. The OR detector, therefore, was used only for the confirmation of the retention time of the optical isomers and the determination; this detector can identify an optical isomer without having to prepare standard, but it was not suitable for analyzing urine from the standpoint of sensitivity. Therefore, the UV detector was thereafter used for the simultaneous analysis of MBDB and BDB enantiomers in rat urine.

Monitoring of MBDB enantiomers in rat urine

Time-lapse changes in the (+)- and (−)-isomers of unchanged MBDB and the metabolite, BDB, in the rat urine found over six intervals, 0–4, 4–8, 8–12, 12–16, 16–18, and 20–24 h, are shown in Table II. After the oral administration of racemic MBDB, both MBDB and BDB were detected in all urine specimens. The urinary excretion of MBDB was high in the urine by 12 h after the administration (between 9.1 ± 1.6% and 9.6 ± 1.6%). BDB showed a high excretion rate in the urine collected from 8 to 12 h (1.7 ± 0.3%). The majority of the two substances were excreted within 24 h. The excreted amount of MBDB and BDB was 34.7 ± 2.8% of the administered dose for MBDB and 4.9 ± 1.0% for BDB, respectively. The total excretion rate of the two substances was 39.6 ± 3.5% (n = 5). The amount detected corresponded to 2559.7 ± 251.3 μg, and the concentration was 268.4 ± 53.9 μg/mL for the total of urinary output to 24 h the concentration was 9.8 ± 1.9 mL. The ratio of the MBDB versus the BDB excreted over six intervals was between 18.9 ± 1.3 and 6.1 ± 4.0. On the other hand, the urinary excretion of (+)-MBDB was somewhat higher than that of (−)-MBDB, and the difference in the excreted amount between the two isomers was statistically significant in the urine from 4 to 20 h (p < 0.05), as shown in Table II. The (+)- and (−)-isomers of MBDB excretions were 17.6 ± 1.4% (119.1 ± 22.1 μg/mL) and 17.1 ± 1.5% (115.4 ± 21.5 μg/mL) within 24 h, respectively. The stereoselective disposition to MBDB was remarkable in the urine from 4 to 20 h. As for BDB, the urinary excretion of (+)-BDB was also higher than that of (−)-BDB at all intervals. The (+)- and (−)-isomers of BDB was 2.9 ± 0.6% (20.0 ± 7.2 μg/mL) and

<table>
<thead>
<tr>
<th>Collection</th>
<th>Enantiomer ratio (−/+</th>
<th>MBDB (M)</th>
<th>BDB (B)</th>
<th>M+B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–4</td>
<td>0.98 ± 0.01</td>
<td>0.68 ± 0.03</td>
<td>0.98 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>4–8</td>
<td>0.98 ± 0.01</td>
<td>0.70 ± 0.04</td>
<td>0.95 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>8–12</td>
<td>0.96 ± 0.02</td>
<td>0.68 ± 0.11</td>
<td>0.91 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>12–16</td>
<td>0.93 ± 0.03</td>
<td>0.76 ± 0.03</td>
<td>0.89 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>16–20</td>
<td>0.85 ± 0.07</td>
<td>0.78 ± 0.02</td>
<td>0.84 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>20–24</td>
<td>0.88 ± 0.09</td>
<td>0.69 ± 0.06</td>
<td>0.85 ± 0.09</td>
<td></td>
</tr>
<tr>
<td>Total**</td>
<td>0.97 ± 0.01</td>
<td>0.70 ± 0.06</td>
<td>0.93 ± 0.02</td>
<td></td>
</tr>
</tbody>
</table>

* The (−)-form/(+)-form ratio of racemic MBDB used is 1.01 ± 0.01 (n = 5).
* n = 5.
* Values are the average ratios of the sum of the (−) form to the sum of the (+) form of MBDB and BDB found in each collection time.
* Not significant versus the enantiomer ratio (−/+ of racemic MBDB used (Student's t-test).
* p < 0.05 versus the enantiomer ratio (−/+ of racemic MBDB used (Student's t-test).
* Values are the average ratios of the sum of the (−) form to the sum of the (+) form of MBDB and BDB excreted over 24 h.

Table III. Time-Lapse Changes in Enantiomer Ratio (−)-form/(+)-form of Unchanged MBDB and the N-Demethylated Substance, BDB, in Rat Urine Specimens after its Oral Administration of Racemic MBDB*
Changes in the enantiomer ratio (-/+) of racemic MBDB

Changes in the enantiomer ratio (-/+) of racemic MBDB, which was calculated from the excreted dose of the (+)-isomer and the (-)-isomer of unchanged MBDB and the N-demethylated metabolite, BDB, at six time intervals, are shown in Table III. After the administration, the ratios (-/+) of unchanged MBDB in urine specimens stored up to 4 h were almost the same as those of the original racemic MBDB used: the ratio (-/+) of the original MBDB was 1.01 ± 0.01. The ratios (-/+) for the other five intervals, 4–8, 8–12, 12–16, 16–20, and 20–24 h, decreased gradually with time. They changed from 0.98 to 0.88 within 24 h (p < 0.05). The ratio (-/+) of unchanged MBDB in the urine excreted up to 24 h was 0.97 ± 0.01. This value was obviously different from that of the original racemic MBDB used (p < 0.05). On the other hand, the ratio (-/+) of BDB had a much lower value than those of unchanged MBDB at all time intervals examined (p < 0.05). This was observed from 4 h urine after the administration of racemic MBDB. The ratio (-/+) was 0.68 ± 0.03, and the value increased to 0.78 ± 0.02 with time by 20 h, as shown in Table III. Thereafter, the value decreased to 0.69 ± 0.06 in 24 h urine. The total ratio (-/+) of MBDB and BDB was also significantly lower than the enantiomer ratio (1.00) of original racemic MBDB used in all intervals (p < 0.05), and the ratio changed from 0.98 to 0.85 within 24 h.

Discussion

Abuse of "Entactogen" MBDB, assigned for the first time by Nichols et al. (1), has permeated Western countries and Japan because of its unique pharmacological properties (2,5,6). This drug has already been found in the urine of MBDB users, and the analysis is being done in the field of forensic chemistry and toxicology (9,11,15). This drug is excreted not only in the urine but also in the saliva and sweat after its oral administration to human subjects (7). The intact parent drug (MBDB) and its metabolite BDB are excreted in the biological fluids within 36 h after a single oral administration (100 mg) (7). The urinary data of humans showed that more MBDB was excreted than BDB, and the urinary excretion of MBDB and BDB showed high peaks at 4 h and 22 h, respectively. The results also indicated that the ratio of MBDB to BDB excreted decreased gradually from 69.1 to 1.4 up to 24 h.

In our results, although the urinary excretion of unchanged MBDB in the rat was higher than that of the metabolite, BDB, as in the human subjects (7), the urinary excretion pattern of MBDB and BDB in the rat did not agree with that of human subjects. The highest excretion of MBDB after the oral administration of MBDB (30 mg/kg) was at 4 h [about 9.6% (235.2 ± 67.4 µg/mL)], but not at 22 h, whereas that of BDB was 8 to 12 h [about 1.7% (115.3 ± 64.2 µg/mL)]. On the other hand, the ratio of MBDB to BDB excreted up to 24 h in the rat was also obviously different from those of humans (7): it was 18.9 to 6.1. Therefore, the disagreement between the rat and the human in urinary excretion kinetics of MBDB and BDB may be due to the difference in administered doses, species and/or metabolic rate.

Our data obtained from the rat suggests that the N-demethylation occurs more readily in the rat than in humans because the rate of MBDB to BDB excreted up to 24 h was about 7.1 in the rat, and it was about 16.1 in humans (7). Furthermore, the conversion to other metabolites than BDB was also expected from the difference in the ratio and the excreted amount of MBDB and BDB between the rat and the human urine specimen. Although the urinary excretion kinetics of MBDB metabolites in the human and the rat has not been described in detail, so far only the methylated dihydroxy-products (13) have been demonstrated in the MBDB users. The metabolites may be the major substances in the rat, so the MBDB/BDB ratio of the rat can be expected to be lower than that of human. It is likely that the reaction to the methylated dihydroxy-products is faster than that to the N-demethylation. Therefore, the excretion rate of BDB would be lower than that of MBDB: 34.7% (234.5 ± 43.6 µg/mL) for MBDB versus 4.9% (33.8 ± 11.0 µg/mL) for BDB in 24 h. Similar findings are observed in the metabolic study of 3,4-dimethylenedioxyamphetamine (MDA) analogues (13,16) and amphetamine (AMP) analogues (17,18); the excretion rate of N-dealkylated substance (MDA analogue or AMP analogue) is lower than that of the intact parent drug.

On the other hand, the optically active type of abused MBDB is thought to be a racemate (1,14). Our study revealed that the urinary excretion kinetic of racemic MBDB differed considerably from that of racemic MDA and MDMA (16). Furthermore, it was shown clearly that the urinary excretion kinetics of MBDB enantiomers is similar to that of methamphetamine (MAMP) (18) and ethylamphetamine (EAMP) (19). In other words, the (+)-isomer of unchanged MBDB and the metabolite, BDB, were excreted a little more than its (-)-isomer in all intervals examined, and the enantiomer ratios (+/-) of MBDB between 4 h and 24 h were lower than that (1.00) of original racemic MBDB used: it was 0.98 to 0.88. These results were clearly different from those of racemic MDA (16) and racemic MDMA (16), which have hallucinogenic activity (1,20). Each ratio (+/-) of racemic MDA and racemic MDMA in the rat was either 1.00 or more in the urine: the ratios (+/-) were 1.17 to 2.29 for MDA and 1.45 to 2.29 for MDMA. By contrast, the ratio (+/-) of racemic MAMP (18) and racemic EAMP (19), which has central nervous system (CNS) stimulant effect (21), was 1.00 or less: the ratio was 0.63 to 0.37 for MAMP and 0.44 to 0.17 for EAMP. Therefore, it is likely that not only the metabolic rate but also the pharmacological effect may contribute to the difference
in the stereoselective disposition in vivo. MBDB and BDB abolish or attenuate hallucinogenic activity (1,2, and the pharmacological effect of (+)-isomer is more potent than that of (-)-isomer (22). This finding is obviously different from that of MDA and MDMA: hallucinogenic effect is found in (-)-enantiomer and stimulant effect in (+)-enantiomer (20). When MBDB was compared with MDA (16) and MDMA (16), the stereoselective disposition was obviously different as mentioned, and it was similar to that of MAMP (18) and EAMP (19), for which the CNS stimulant effect of (+)-enantiomer is more potent than that of the (-)-enantiomer (21). Although the pharmacological effect of MDEA enantiomers has not been clarified yet, it is presumed that MDEA has no hallucination effect (1,23). Other reports suggest that MDEA is a psychoactive substance, which takes an intermediate position between stimulants and the hallucinogens (24). These findings are obviously different from that of MDA (20) and MDMA (1). One can expect that the urine excretion kinetics of MDEA enantiomer is different from that of them. Furthermore, the stereoselective disposition of racemic MDEA corresponded to that of racemic MBDB in this study; the ratio (+/-) of MDEA is 0.93 to 0.83 (16).

Thus, our previous study revealed that a stereoselective disposition to racemic MBDB was different from that of MDA and MDMA and was similar to that of MDEA, MAMP, and EAMP. Our study has succeeded in the simultaneous enantiomer monitoring of clandestine MBDB, and it could contribute to police forensic chemistry and toxicological research on MBDB enantiomers.

References


Manuscript received February 5, 2001; revision received August 14, 2001.