Excretory Profile of 4-Bromo-2,5-dimethoxyphenethylamine (2C-B) in Rat

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The urinary and faecal metabolic profiles of 4-bromo-2,5-dimethoxyphenethylamine (2C-B) in rat were investigated. Male Wistar rats were administrated 10 mg/kg of 2C-B hydrochloride orally, and the urine and faeces were collected 0–24 and 24–48 hr after administration. The samples were processed by liquid-liquid extraction, and the extracts analyzed by GC/MS, after derivatization. The major metabolite excreted into the urine was 5-*O*-desmethyl-*N*-acetyl-2C-B, comprising 13.2% of the administrated dose. Other metabolites detected in the urinary extracts were 2-*O*-desmethyl-*N*-acetyl-2C-B (5.8%), 2-*O*-desmethyl-2C-B (3.5%), carboxylic acid compound (1.9%), 5-*O*-desmethyl-2C-B (1.2%) and an alcoholic compound (0.8%). Only 0.2% of the administrated 2C-B was excreted into the urine in its original form. On the other hand, only 5-*O*-desmethyl-*N*-acetyl-2C-B and 2-*O*-desmethyl-*N*-acetyl-2C-B were detected in the faecal extracts. The major fraction of the urinary metabolites that contained a hydroxy group were recovered as conjugates.

Key words —— 4-bromo-2,5-dimethoxyphenethylamine, metabolite, rat, urine, faeces, quantitation

INTRODUCTION

4-Bromo-2,5-dimethoxyphenethylamine (2C-B) is a drug which possesses psychoactive properties.[1,2] The ingestion of 4–30 mg of 2C-B induces euphoria along with an increased sensitivity to visual, auditory, olfactory and tacticle sensations. Currently, 2C-B is under legislative control in many countries, including Japan.

2C-B was first synthesized in 1974[1] and entered the drug market in the 1980's,[3] but little information is available concerning its metabolic fate. In a previous study, we reported on the identification of six metabolites of 2C-B in the urine of rats had been administrated 2C-B hydrochloride.[4] We wish to report herein on the quantitative analysis of these metabolites in urine and feaces as well as that of unchanged 2C-B 48 hr after administration, in an attempt to clarify the metabolic behavior of 2C-B.

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MATERIALS AND METHODS

Materials —— Authentic standards of 2C-B and its identified metabolites were synthesized in our laboratory as described previously.[4] β-Glucuronidase/aryl sulfatase (from *Helix pomatia*; β-glucuronidase, 6.76 units/ml; aryl sulfatase, 2.08 units/ml) was purchased from Calbiochem-Novabiochem Co. Ltd (La Jolla, CA, U.S.A.). All other chemicals used were of analytical grade.

Drug Administration and Urine Sampling —— Four male Wistar rats were orally administrated 10 mg/kg of 2C-B hydrochloride and placed in metabolic cages. The 0–24 and 24–48 hr urinary and faecal fractions were collected and stored at –20°C until used for analysis.

Extraction of the Metabolites and Sample Preparation —— To one half ml of urine, an equal volume of 0.2 M phosphate buffer (pH 7.0) was added and the pH adjusted to 7.0 by adding 0.1 M hydrochloric acid. A 5 μl portion of β-glucuronidase/aryl sulfatase was added to the urinary sample followed by incubation at 37°C for 24 hr. After the addition of 2,5-dimethoxy-4-propylthiophenethylamine and 2,5-dimethoxyphenylacetic acid as internal standards, the pH was adjusted to 9 by adding 10% sodium carbonate solution, and the solution was extracted with chloroform/isopropanol (9 : 1, v/v) (basic fraction). After adjusting the pH to 2 by adding 3 M hydrochloric acid, the remaining aqueous layer was extracted with diethylether (acidic fraction). For the quantitation of unconjugated forms, in the basic fraction, the step involving β-glucuronidase/aryl sulfatase hydrolysis was omitted. Each fraction was de-

Table 1. Recovery (%) of 2C-B and Its Known Metabolites from Spiked Urine Sample

Metabolite	Basic fraction	Acidic fraction
Alcoholic compound	91.5 ± 6.6	—
Carboxylic acid compound	—	95.2 ± 6.8
2-*O*-Desmethyl-2C-B	86.0 ± 5.5	—
5-*O*-Desmethyl-2C-B	78.7 ± 5.0	—
2-*O*-Desmethyl-*N*-acetyl-2C-B	96.4 ± 6.4	—
5-*O*-Desmethyl-*N*-acetyl-2C-B	93.5 ± 5.6	—
2C-B	82.3 ± 1.9	—

Blank urine samples were spiked with each compound at a concentration of 1 μg/ml. Data represent the mean ± S.D. of four determinations.

hydrated with anhydrous sodium sulfate and evaporated to dryness under vacuum. The residue of basic fraction was dissolved in 100 μl of tetrahydrofuran (THF) containing 10% pyridine and 10% *n*-butyric anhydride for butyryl derivatization, prior to GC/MS analysis. The residue of the acidic fraction, dissolved in 50 μl of acetonitrile and 50 μl of *N*,*O*-bistrimethylsilyl-trifluoroacetamide (BSTFA), was heated at 80°C for 30 min for trimethylsilyl (TMS) derivatization, and was then analyzed by GC/MS.

A 200 mg sample of faecal material was suspended in 8 ml of methanol-water (1 : 1, v/v), sonicated for 10 min, and then shaken for 20 min on a mechanical shaker. After centrifugation at 3000 rpm for 10 min, the supernatant was collected and evaporated to dryness under vacuum. The residue was suspended in 1 ml of 0.1 M phosphate buffer (pH 7.0), then extracted and analyzed by the same method as was used for the urine. The remaining aqueous layer was hydrolyzed by β-glucuronidase/arylsulfatase, then extracted again, and analyzed by the same method.

GC/MS —— GC/MS analysis was performed using a Finnigan · MAT GCQ (San Jose, CA, U.S.A.) equipped with a DB-5ms capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness, J&W scientific, Folsom, CA, U.S.A.). The oven temperature was held at 120°C for 1 min following injection and then programmed to 280°C at a rate of 15°C/min. The injection port and interface temperature were set at 250°C and 275°C, respectively. Helium was used as carrier gas at a flow rate of 1 ml/min. The mass spectrometer was operated under the electron ionization (EI) mode at an ionization energy of 70 eV. Samples were injected in the splitless mode.

Calibration Curve —— Authentic standards of 2C-B and its known metabolites were added to the urinary or faecal samples as controls, and processed as described above to obtain the calibration curves. Excellent linearity was obtained over the concentration range 0.1–10 μg/ml with a correlation coefficient of 0.99.

RESULTS AND DISCUSSION

Urinary and faecal samples obtained from the rats that had been orally administrated 2C-B were processed by liquid-liquid extraction, and the metabolites of 2C-B in the extracts were quantitatively analyzed by GC/MS after derivertization.

Table 1 shows the recoveries of 2C-B and its known metabolites from urinary samples spiked with these reference compounds. 2C-B and five metabolites were found in the basic fraction, while the carboxylic acid compound was found in the acidic fraction. Satisfactory recoveries were obtained for the respective compounds, ranging 78.7–96.4%.

The excretory profile of 2C-B into urine is summarized in Table 2. The excretion of unchanged 2C-B and its known metabolites was almost complete 24 hr after administration. Small amounts of 5-*O*-desmethyl-*N*-acetyl-2C-B and 2-*O*-desmethyl-*N*-acetyl-2C-B were excreted in the 24–48 hr period. The main metabolite detected in the urinary sample was 5-*O*-desmethyl-*N*-acetyl-2C-B, accounting for 13.2% of the dose in 48 hr. The amounts of the other metabolites were, in descending order, 2-*O*-desmethyl-*N*-acetyl-2C-B (5.8%), 2-*O*-desmethyl-2C-B (3.5%), carboxylic acid compound (1.9%), 5-*O*-desmethyl-2C-B (1.2%) and alcoholic compound (0.8%). The recovery of unchanged 2C-B was as low as 0.2%. The metabolites with a hydroxy group were excreted mainly as conjugates. For example, the recovery of the free form of 5-*O*-desmethyl-*N*-acetyl-2C-B was only 0.2% of the administrated dose in comparison with the total recovery of 13.2%, and the alcoholic compound and 5-*O*-desmethyl-2C-B

Table 2. Excretion of 2C-B Metabolites in the Urine of the Rat

	% of the primed dose of 2C-B			
	0–24 hr fraction		24–48 hr fraction	
Metabolite	total	free	total	free
Alcoholic compound	0.8 ± 0.3	ND	ND	ND
Carboxylic acid compound	1.9 ± 0.5	—	ND	—
2-*O*-Desmethyl-2C-B	3.5 ± 1.5	1.9 ± 0.7	ND	ND
5-*O*-Desmethyl-2C-B	1.2 ± 0.4	ND	ND	ND
2-*O*-Desmethyl-*N*-acetyl-2C-B	5.8 ± 1.0	0.6 ± 0.4	0.03 ± 0.03	ND
5-*O*-Desmethyl-*N*-acetyl-2C-B	13.1 ± 0.5	0.2 ± 0.1	0.1 ± 0.08	ND
Unchanged 2C-B	0.2 ± 0.08	0.2 ± 0.08	ND	ND

Data represent the mean ± S.D. of four rats. ND: not detected.

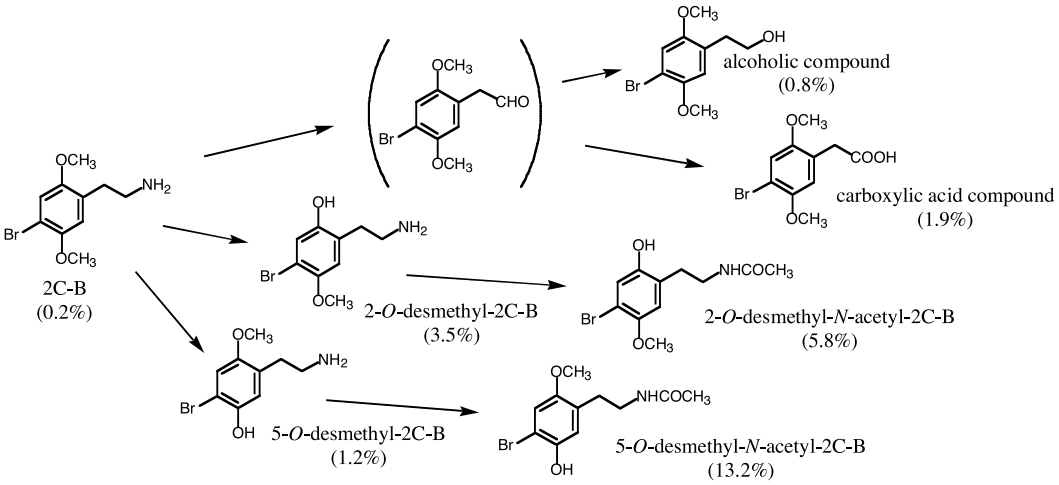

Fig. 1. Proposed Metabolic Pathways for 2C-B in Rat
The numbers in parentheses represent the amount of each metabolite excreted in the rat urine 48 hr after administration.

could not be detected when the enzymatic hydrolysis step was omitted. The case of 2-*O*-desmethyl-2C-B was, however, exceptional because the unconjugated form was more abundant than the conjugated one.

Since it is well known that many types of drugs and their metabolites are excreted in faeces via the bile[5] as well as the urine, the excretory profile of 2C-B was also examined using faecal samples. Although the metabolites of 2C-B, irrespective of conjugation, have molecular weights sufficient for excretion in bile, only small amounts of 5-*O*-desmethyl-*N*-acetyl-2C-B (1.7% of dose) and 2-*O*-desmethyl-*N*-acetyl-2C-B (0.7%) were detected in the 0–24 hr faecal fractions.

Based on these observations as well as those in our previous paper, the metabolic fate of 2C-B in the rat can be tentatively depicted in Fig. 1. At least two metabolic pathways for 2C-B appear to exist. The first leads to transformation to alcoholic or carboxylic acid compounds, and the second leads to the formation of *O*-desmethyl or *O*-desmethyl-*N*-acetyl derivatives. Taking the quantitative data obtained in this study into consideration, the latter pathway appears to be dominant in rats. However, the total amount of metabolites identified in urine and faeces, as of today, was about 30% of the total, leaving the remaining 70% still missing. Extremely polar (in another words, hydrophilic) compounds represent likely candidates for the missing compounds in this study. In order to add to our knowledge concerning the metabolic fate of 2C-B, especially undetectable compounds, further studies are currently in progress in our laboratory.

REFERENCES

1) Shulgin, A. T. and Carter, M. F. (1975) Centrally active phenethylamines. *Psychopharmacol. Commun.*, **1**, 93–98.
2) Shulgin, A. and Shulgin, A. (1992) *Pihkal, A Chemical Love Story*, Transform Press, Berkeley, CA, U.S.A.
3) Ragan, F. A., Hite, S. A., Samuels, M. S. and Garey, R. E. (1985) 4-Bromo-2,5-dimethoxyphenethylamine: identification of a new street drug. *J. Anal. Toxicol.*, **9**, 91–93.
4) Kanamori, T., Inoue, H., Iwata, Y., Ohmae, Y. and Kishi, T. (2002) In vivo metabolism of 4-bromo-2,5-dimethoxyphenethylamine (2C-B) in the rat: identification of urinary metabolites. *J. Anal. Toxicol.*, **26**, 61–66.
5) La Du, B. N., Mandel, H. G. and Way, E. L. (1971) *Fundamentals of Drug Metabolism and Drug Disposition*, The Williams & Wilkins Company, Baltimore, U.S.A.