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Disposition of 4-bromo-2,5-dimethoxyphenethylamine (2C-B) and its metabolite 4-bromo-2-hydroxy-5-methoxyphenethylamine in rats after subcutaneous administration

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Abstract

The psychedelic compound 4-bromo-2,5-dimethoxyphenethylamine (2C-B) has appeared as an agent in drug abuse or overdose cases in humans. The human pharmacokinetics of this drug is unknown and only partial information is available on its metabolites. Our experimental study was focused on the disposition and kinetic profile of 2C-B in rats after subcutaneous administration using a GC–MS validated method. One of the major metabolites 4-bromo-2-hydroxy-5-methoxyphenethylamine (2H5M-BPEA) was confirmed in rat tissues of lung, brain, liver and was quantitatively evaluated as well. The disposition of 2C-B was characterized by its estimated half-life 1.1 h and estimated volume of distribution 16 L/kg. The lung susceptibility for drug retention and gradual temporal release parallel to the brain were ascertained. The drug penetrating the blood/brain barrier was without significant delay. 2C-B brain to serum ratio attained a maximum value of 13.9 and remained over the value of 6.5 to the end of our observation (6 h after the dose). The distribution of the hydroxylated metabolite 2H5M-BPEA into the lipophilic brain tissue was less efficient in relation to the parent compound. The kinetics of the drug partitioning between blood to brain may be important for the subsequent assessment of its psychotropic or toxic effects.

Keywords: 2C-B; 4-bromo-2,5-dimethoxyphenethylamine; Pharmacokinetics; Disposition; Metabolites; Rats

1. Introduction

2C-B or BDMPEA (4-bromo-2,5-dimethoxyphenethylamine) is a psychoactive agent related to both the mescaline-like psychedelics and the amphetamine-like stimulants. The synthesis of this compound has been described by Shulgin who also tested its pharmacological effects on himself and a group of volunteers (Shulgin and Carter, 1975; Shulgin and Shulgin, 1991). 2C-B is a potential drug of abuse and it has appeared as an alternative or a complementary drug to Ecstasy on the illegal drug market; earlier it was also sold via the Internet and nowadays it appears especially on the dance scene in the form of various tablets with questionable composition and potential risk of intoxication (Giroud et al., 1998; de Boer et al., 1999a; de Boer and Bosman, 2004; Laing and Siegel, 2003a). This drug is also known under a variety of street names such as "Nexus, Venus, Bromo, Erox, or Synergy".

2C-B possesses an affinity towards various central serotonergic and adrenergic receptors (Glennon and Anderson, 1980; Glennon et al., 1988, 1992; Villalobos et al., 2004; Lobos et al., 1992; Monte et al., 1996; Neuvonen et al., 2006). Activation of the 5-HT_{2A} (serotonin 2A) receptors by an agonist ligand has been established to be bound with hallucinogenic effect (e.g. Monte et al., 1996; Villalobos et al., 2004; Neuvonen et al., 2006). In humans, 2C-B is usually consumed orally in the range of 4–30 mg dosages, which can produce considerable euphoria with increased receptiveness of the visual, auditory, olfactory, and tactile sensations. Lower doses (4–10 mg) induce entactogenic-stimulating effects while higher doses (10–20 mg) induce psychedelic effects along with frightening hallucinations and some sympathomimetic effects such as tachycardia, hypertension and hyperthermia (Shulgin and Carter, 1975; Shulgin and Shulgin, 1991; Carmo et al., 2005). Laing and Siegel (2003b) described its hallucinogenic potency as being 16 times stronger

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than mescaline and Shulgin and Carter (1975) have described that its potency is about one tenth of its three carbon counterpart 4-bromo-2,5-dimethoxyamphetamine (DOB). The average duration of the effects in humans after 2C-B ingested dose ranges between 4 and 8 h depending on the dose and the susceptibility of an individual (Shulgin and Shulgin, 1991; Karch, 2002).

At present, the pharmacokinetics of 2C-B based on controlled study is unknown. To clarify the disposition of this drug in organism related to time after the dose may be important from the perspective of successful detection and correct assessment of an intoxication or drug abuse and from the perspective of understanding and explanation of its psychotropic effects. In the case of 2C-B, the biotransformation scheme related to mescaline can be assumed. It involves *O*-demethylation of methoxygroups of the aromatic ring, beta-oxidation of the alkyl chain and oxidative deamination involving different enzyme systems (Scheline, 1978; Moffat et al., 2004; Charalampous et al., 1966). Today, results of some biotransformation studies of 2C-B on experimental animal models are available (Kanamori et al., 2002, 2003, 2005; Carmo et al., 2005, 2004; Theobald et al., 2007). De Boer et al. also reported on the human metabolites found in the urine specimen of a subject abusing 2C-B (de Boer et al., 1998; de Boer et al., 1999b). In this single human urine sample, the presence of the parent form and the 4-bromo-2,5-dimethoxyphenylacetic acid was confirmed using GC–MS with reference standards. The formation of some other metabolites was also indicated, however, they could not be identified unambiguously due to the lack of reference standards. The systematic clinical study of this drug with human volunteers remains restricted for ethical reasons and therefore only various experimental animal studies have been performed to date. In the experimental study with hepatocytes of six species including humans (Carmo et al., 2005), the metabolic pathways of 2C-B was constructed with the GC–MS identification of the metabolite 4-bromo-2,5-dimethoxyphenylacetic acid in all species. The other metabolites formed by combination of oxidative deamination and *O*-demethylation were indicated too considering the mass spectrometry fragmentation mechanisms.

Our study was focused on quantitative assessment of the kinetic profiles of 2C-B and its prevailing *O*-demethylated metabolite in serum, brain, liver and lung tissues of experimental rats after subcutaneous administration. The identification of the monodemethylated metabolites and evaluation of their abundance in serum and tissues was based on synthesized reference substances of 4-bromo-2-methoxy-5-hydroxyphenethylamine (2M5H-BPEA) and 4-bromo-2-hydroxy-5-methoxyphenethylamine (2H5M-BPEA). The assessment of other potential metabolites of 2C-B in samples under our study cannot be performed fully as the reference standards were not available.

2. Materials and methods

2.1. Chemicals and reference standards

Chemicals used were of analytical grade. Extraction discs from SPEC PLUS 3 ML DAU, Ansys Technologies were provided by Amedis Ltd. Prague. 2-methylamino-1-(3,4-methylenedioxyphenyl)-butane hydrochloride (MBDB) used as internal standard was kindly provided by UNDCP, Vienna. The reference analytical standard 4-bromo-2,5-dimethoxyphenylethylamine hydrochloride (2C-B) was provided by Sigma–Aldrich Ltd., Prague (purity > 99%).

Other reference standards, both *O*-monodesmethyl metabolites (2M5H-BPEA and 2H5M-BPEA) were synthesized according to Kanamori et al. (2002) with ^{1}H and ^{13}C NMR structural verification and provided as hydrochlorides by Pharmaceutical Faculty of Charles University in Hradec Králové with estimated purity of 81 and 80%.

2.2. Animals and drug administration

All experiments were performed in compliance with international guidelines and laws governing animal studies in the Czech Republic. Male rats Wistar specific pathogen free (SPF; Velaz Ltd., Prague) weighing 0.230–0.250 kg were housed individually in cages. They had free access to water and food and were kept under a 12-h day and night cycle. They were administered a 50 mg/kg bolus dose of 2C-B hydrochloride dissolved in physiological solution in a volume of 2 ml/kg subcutaneously. The animals were sacrificed at 30, 60, 120, and 360 min after dosing (10 animals at each time interval) and serum, brain, liver and lung samples were collected and stored at −20 °C until analyses.

2.3. Sample preparation

2.3.1. Serum pretreatment

0.5 ml aliquot of rat serum was transferred to a labeled tube. Internal standard (400 ng/ml MBDB) and 1 ml 0.1 M phosphate buffer (pH 6) were added and mixed.

2.3.2. Tissue pretreatment

1 g of tissue (brain, liver or lung) was homogenized with 4 ml methanol and internal standard aliquot (400 ng/g MBDB for brain; 2000 ng/g MBDB for liver and lung). The specimen was mixed and treated for 20 min in an ultrasonic bath followed by water freezing (20 min) and centrifugation (5 min at 4000 rpm). 2 ml of supernatant were transferred to a clean glass test tube and evaporated. The residue was reconstituted by adding 0.1 ml methanol and 1 ml of 0.1 M phosphate buffer (pH 6).

2.3.3. Solid phase extraction procedure (SPE)

A pretreated specimen with buffer and internal standard was transferred to the SPE disc preconditioned with 1 ml methanol and 1 ml phosphate buffer (pH 6). After sample application the disc was washed with 1 ml distilled water, 1 ml 0.1 M HCl and 1 ml methanol and then finally dried with air for 5 min. Analytes were eluted three times with 1 ml of the fresh mixture of dichlormethane/2-propanol/ammonium hydroxide (25%), 80/20/4, v/v/v. The combined eluates were evaporated to dryness under an air stream at 40 °C and derivatized.

2.3.4. Derivatization

The chemical derivatization by acetylation was performed by treatment of dry residues with 200 μl of the acetylation mixture (acetic acid anhydride/pyridine 10/1) at 60 °C for 30 min. The derivatized samples were then allowed to cool to ambient temperature prior to evaporation of excess acetylation agent. The residues were reconstituted in 100 μl ethylacetate and 1 μl aliquot was analyzed using GC–MS.

2.4. GC–MS conditions

The Hewlett-Packard GC–MS system HP 6890-5973 (Agilent, Waldbronn Germany) equipped with an autosampler, splitless injector and capillary HP5-MS 30 m × 0.25 mm × 0.25 μm were used for the analyses. Helium was used as the carrier gas at the flow rate of 1.0 ml/min. The oven temperature was programmed starting from 85 °C, held for 2 min, then with 30 °C/min up to 150 °C, then with 15 °C/min up to 250 °C and held for 10 min. The injector and the transfer line were operated at 250 °C. Ionization was carried out in electron impact (EI) at 70 eV. The selected ion monitoring (SIM) mode was used; quantifying and monitored ions were for the internal standard MBDB

(249, 176, 135, 114, 72), for 2C-B (301, 242, 229, 199, 148), for 2H5M-BPEA (329, 287, 228, 187, 148, 134, 77)—the underlined ions indicate the quantifying ions.

2.5. Method validation and determination of 2C-B and 2H5M-BPEA concentrations

The GC–MS toxicological methods were developed and validated in compliance to international standards (Peters and Maurer, 2002; Peters et al., 2007) to determine the 2C-B and its main metabolite 2H5M-BPEA concentrations in rat samples under the operating conditions described above. The calibration was based on linear regression analysis using the ratio of peak areas of the analyte to the internal standard. Blank rat serum and tissue samples collected from laboratory male Wistar rats were used for calibration and validation purposes. Selectivity was checked with samples obtained from six different animals. 2C-B and 2H5M-BPEA calibrators were obtained by adding appropriate amounts of 2C-B, 2H5M-BPEA and internal standard to blank matrices to imitate the concentration corresponding to values expected in real samples. Internal standard addition was 400 ng/ml for serum, 400 ng/g for brain and 2000 ng/g for lung and liver. Six point calibrators with two replicates for each level were used.

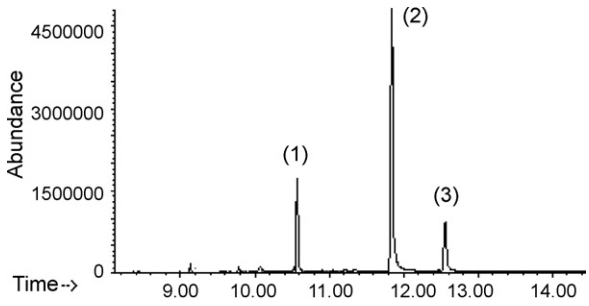

Fig. 1. Chromatogram corresponding to an authentic rat liver sample 60 min after 2C-B subcutaneous administration: (1) MBDB.AC, internal standard; (2) 2C-B.AC, parent drug; (3) 2H5MBPEA.2AC, metabolite.

The limits of detection and quantification were determined separately using spiked calibrators to lower concentrations in the range of 30–200 ng/ml (or ng/g) of both analytes.

The control samples to evaluate precision and accuracy of both analytes were prepared independently at three concentration levels in respect to the real

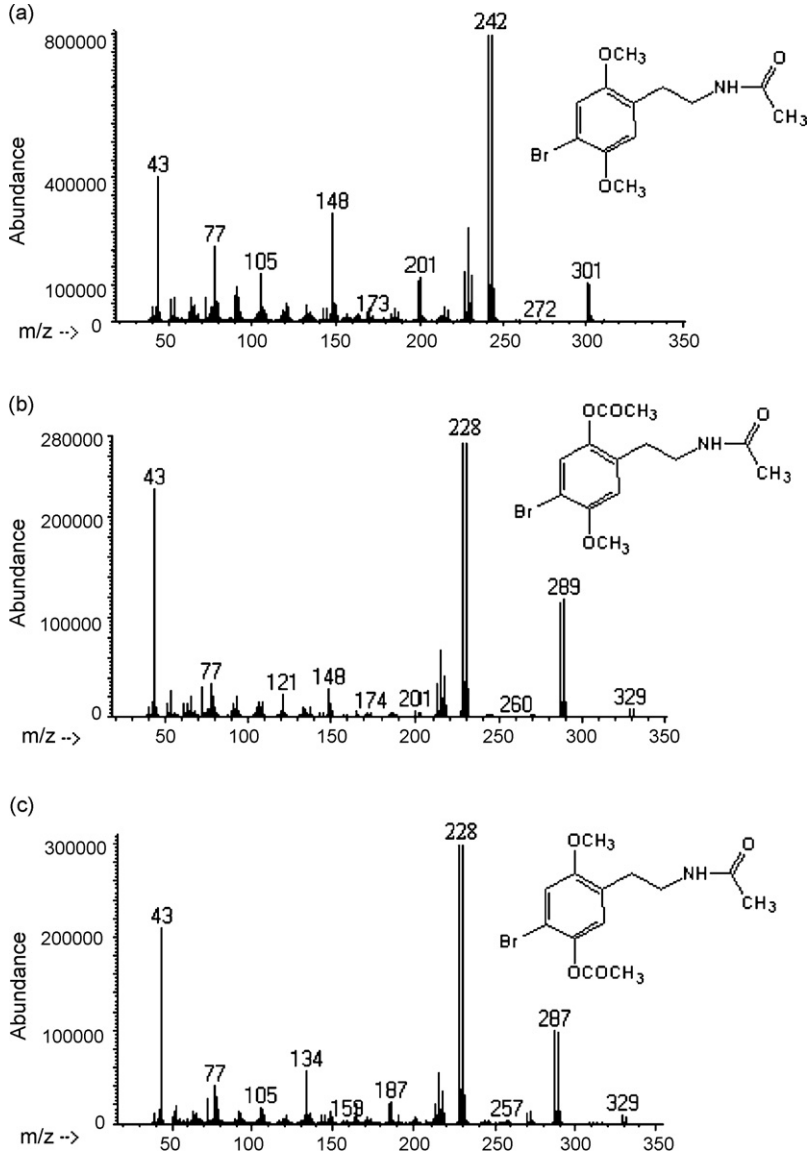

Fig. 2. Typical mass spectra of reference standards of acetylated 2C-B and its expected *O*-desmethyl metabolites: (a) 2C-B.AC, (b) 2H5M-BPEA.2AC, (c) 5H2M-BPEA.2AC.

Table 1
Linearity of calibration of GC–MS assay in various rat matrices: regression coefficient (R^2), limits of detection (LOD) and lower limit of quantification (LLOQ)

ANALYTE	SAMPLE	Calibration range (ng/ml; ng/g)	R^2	LLOQ (S/N > 8)	LOD (S/N > 3)
2C-B	Serum	100–3000	0.999	100 ng/ml	50 ng/ml
	Brain	100–20,000	0.998	70 ng/g	30 ng/g
	Liver	100–9000	0.999	70 ng/g	30 ng/g
	Lung	100–40,000	0.996	100 ng/g	50 ng/g
2H5M-BPEA	Serum	200–3000	0.996	200 ng/ml	120 ng/ml
	Brain	130–6000	0.996	120 ng/g	80 ng/g
	Liver	120–9000	0.999	120 ng/g	80 ng/g
	Lung	120–9000	0.998	120 ng/g	80 ng/g

samples. The precision (repeatability) and accuracy values were evaluated by analyzing six samples per day. Accuracy was calculated as the difference of the mean of determined values ($n = 6$) from the theoretical one related to the theoretical one.

3. Results

3.1. GC–MS method validation parameters

Fig. 1 shows, as an example, the chromatogram related to an authentic rat liver sample obtained 60 min after administration. The GC–MS spectra of acetylated standards of the parent compound 2C-B and both potential *O*-desmethyl metabolites 2H5M-BPEA and 2M5H-BPEA expected in rat samples in our study are presented in Fig. 2.

The GC–MS method developed was found to be selective both for serum and tissues and no interferences with analytes of interest or internal standard were ascertained.

The method linearity was fulfilled in the concentration range given in Table 1 with regression coefficients better than 0.996 both for parent 2C-B and its main metabolite 2H5M-BPEA. The 2C-B limits of detection (LOD), S/N > 3, in serum and tissues were at least 50 ng/ml (ng/g), lower limits of quantification (LLOQ), S/N > 8, at least 100 ng/ml (ng/g) or better. These LOD and LLOQ values for 2H5M-BPEA were in selected tissues 80 ng/g (LOD) and 120 ng/g (LLOQ). These limits were rather higher in the serum, LOD 120 ng/ml and LLOQ 200 ng/ml.

Repeatability of all assays was within 9%, except the value near the LLOQ (11%). Accuracy was found to be within ±11% of the spiked value, except at the LLOQ where higher values were found. The results are summarized in Table 2.

3.2. Structural identification of 2C-B and its O-demethylated metabolites in rat samples

The detection and identification of 2C-B in rat sample extracts was based on direct comparison with the reference substance. The identification and structural confirmation of 2H5M-BPEA as the main metabolite determined in rat serum and tissues after

Table 2
Repeatability and accuracy of the GC–MS assay ($n = 6$)

Analyte	Sample	Spiked concentration (ng/ml; ng/g)	Repeatability (%)	Accuracy (%)
2C-B	Serum	100	3.7	−1.8
		500	3.1	+8.9
		2000	1.8	+0.4
	Brain	100	4.6	−12.9
		2000	2.3	−10.1
		15,000	5.5	+9.9
	Liver	100	7.9	+19.8
		2000	4.3	+2.9
		9000	2.2	−3.0
	Lung	100	4.6	−5.7
		2000	3.8	+3.9
		30,000	2.2	−4.0
2H5M-BPEA	Serum	200	2.4	−10.1
		400	2.2	−0.7
		2000	4.1	+6.5
	Brain	130	6.2	+12.3
		1500	7.3	−9.6
		5000	3.3	−2.2
	Liver	120	10.6	+8.1
		2000	8.3	−1.7
		9000	6.7	+0.8
	Lung	120	10.8	−1.4
		2000	4.6	−8.6
		9000	5.3	+4.9

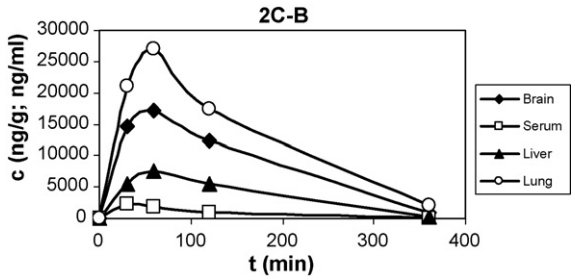

Fig. 3. Temporal distribution characteristic of 2C-B in rat serum, brain, liver and lung after 50 mg/kg s.c. application. Mean values ($n = 10$).

our experimental 2C-B dose, was also based on the accordance with mass spectra and retention of synthesized reference standard. The abundance of the other *O*-demethylated metabolite 2M5H-BPEA (which could be distinguished from the previous one by GC–MS) was negligible in all studied samples and therefore quantitative evaluation could not be performed.

3.3. Temporal disposition profile of 2C-B and 2H5M-BPEA metabolite

All animals tolerated the drug subcutaneous dose 50 mg/kg during 6 h of our kinetic study. The determined kinetic profiles of the parent 2C-B and its main metabolite 2H5M-BPEA found in serum and various rat tissues are presented in the form of mean values ($n = 10$) in Figs. 3 and 4. The values corresponding to individual animals are summarized in Table 3.

Absorption of the parent drug into the blood stream was rapid after the subcutaneous dose; the maximum 2C-B serum concentration (mean c_{max} 2250 ± 253 ng/ml) was attained within 30 min ($t_{max} = 30$ min) as displayed in Fig. 3. The kinetic profiles of 2H5M-BPEA metabolite in various tissues are demonstrated in Fig. 4. The metabolite 2H5M-BPEA could not be detected in serum samples during the whole time interval.

The appearance of the parent 2C-B in all examined tissues was rather delayed related to serum ($t_{max} = 60$ min) but gradually accomplished significantly higher tissue concentrations as expected. Based on the data of this study, the 2C-B abundance in tissues was in the order ranging from lung > brain > liver > serum. The highest tissue concentrations of the parent drug were found in the lung (c_{max} 27028 ± 7777 ng/g) whereas the lowest ones were found in the liver (c_{max} 7485 ± 1534 ng/g), nevertheless these were higher than those in serum. After 6 h, 2C-B abundance in tissues still persisted high above the detection limit (Fig. 3, Table 3).

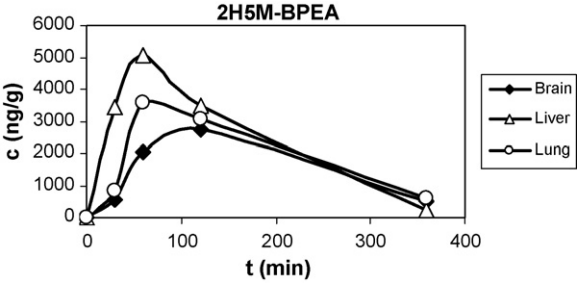

Fig. 4. Temporal distribution characteristic of 2H5M-BPEA in rat brain, liver and lung after 2C-B 50 mg/kg s.c. application. Mean values ($n = 10$). In serum this metabolite was not detected.

The abundance of the metabolite 2H5M-BPEA in tissues reached its maximum within 1 h (liver, lung) or 2 h (brain) after the 2C-B dose (Fig. 4). The concentration levels of more polar metabolite 2H5M-BPEA in the brain and lung were much lower related to the parent compound reaching maximum values of 3761 ± 1744 ng/g (lung) and 2726 ± 938 ng/g (brain) as demonstrated in Fig. 4 and Table 3. In the liver, the important organ for metabolism, the abundance of 2C-B and its metabolite were relatively close. The kinetic curves of both compounds in the liver demonstrate parallel temporal profiles, a gradual rise and then decline, and both curves remained close to each other until the end of our observation.

The kinetic curves of lipophilic 2C-B in serum and all tissues demonstrate significant drug retention in the lung and sufficient influx into the brain. The brain/serum ratio was >6.5 starting at 30 min and was kept above this value throughout the 6 h of our experiment. The 2C-B penetration of the blood–brain barrier was non problematic. The distribution of the hydroxylated metabolite 2H5M-BPEA into the lipophilic brain tissue was less efficient and lung retention less apparent related to the parent drug.

4. Discussion

This study was performed to investigate the temporal disposition of the designer drug of abuse 2C-B, structurally related to amphetamine and mescaline. Concern has been raised because insufficient information is known about 2C-B pharmacokinetics and metabolism which may be important from diagnostic and therapeutic perspective of an intoxication. The experimental design was based on laboratory rats as the human clinical study remains difficult from ethical reasons. The animal model used in our study was rather different from 2C-B abuse common mode in humans, even if unexpected events appear in toxicological practice. The subcutaneous administration was applied to rats to assure reproducible drug bioavailability between animals which is important for toxicokinetic study and the high dose was in favour of easier detection and identification of unknown metabolites.

The results of the kinetic disposition study may be interesting also in the connection with the subsequent assessment of drug effects. The structural features of 2C-B, with halogen substitution at position 4 of the aromatic ring, correspond in an optimal way to the requirements for hallucinogen-like activity as expressed by others (e.g. Monte et al., 1996; Neuvonen et al., 2006). There are many proofs that 2C-B links to hallucinogenic drug activity with its affinity at 5-HT_{2A} receptors that gives better understanding to the effects of psychotropic drugs (Glennon and Anderson, 1980; Glennon et al., 1988, 1992; Villalobos et al., 2004; Lobos et al., 1992; Monte et al., 1996; Neuvonen et al., 2006). The significant drug persistence in the brain ascertained in our experimental rats is a prerequisite for 2C-B psychotropic effects. As 2C-B is the lipophilic psychedelic substance, we expected and found a high concentration in the brain, analo-

Table 3
Temporal concentration profile of 2C-B and its metabolite 2H5M-BPEA in serum, brain, liver and lung of individual rats after 50 mg/kg s.c. 2C-B application

t (min)	2C-B					2H5M-BPEA		
	Animal n.	Serum (ng/ml)	Brain (ng/g)	Liver (ng/g)	Lung (ng/g)	Brain (ng/g)	Liver (ng/g)	Lung (ng/g)
30	1	2188	10,412	5631	17,429	351	3403	132
30	2	2583	21,370	6599	14,881	1150	3311	513
30	3	2092	15,273	7572	258,36	966	5571	536
30	4	2625	14,706	6015	16,542	326	3200	62
30	5	2083	15,318	5553	20,317	853	3897	640
30	6	2208	16,911	4879	21,809	754	3275	500
30	7	1945	14,005	3152	15,662	380	2416	433
30	8	2638	16,115	4449	22,471	410	3962	274
30	9	1962	12,801	6500	20,930	770	2949	178
30	10	2178	9480	3730	34,706	766	2343	432
Mean value (±S.D.)		2250 ± 253	14639 ± 3042	5408 ± 1237	21058 ± 5335	673 ± 273	3433 ± 872	370 ± 185
60	11	1468	21,712	7545	21,929	2174	5642	2055
60	12	2097	24,515	8150	42,555	2567	4993	7120
60	13	2498	24,994	8732	38,443	1426	5392	3631
60	14	2184	11,696	6083	20,237	912	3876	1970
60	15	1706	15,213	7958	24,317	1326	5048	2658
60	16	895	13,568	5747	17,800	1913	5260	6078
60	17	2909	16,035	5985	30,160	3189	4516	5329
60	18	1397	11,820	6658	26,173	2064	4906	3157
60	19	2018	17,404	11,360	31,554	4015	6572	3750
60	20	1751	14,067	6632	17,117	851	4640	1864
Mean value (±S.D.)		1892 ± 523	17102 ± 4493	7485 ± 1534	27028 ± 7777	2043 ± 954	5085 ± 683	3761 ± 1744
120	21	484	8538	3568	14,274	3182	2671	592
120	22	933	13,746	3192	15,688	2605	2157	662
120	23	541	10,979	7129	22,680	1976	5275	2398
120	24	1142	9285	7255	19,947	3190	5484	4289
120	25	770	16,866	8754	25,469	2062	5693	2283
120	26	963	10,926	3516	15,425	4007	2453	2260
120	27	1199	19,094	4851	8701	1509	3797	3603
120	28	638	8882	6346	20,142	1335	3878	3381
120	29	937	8997	5017	8739	3240	3343	2343
120	30	1390	17,307	4570	29,893	4154	2732	5073
Mean value (±S.D.)		900 ± 279	12462 ± 3786	5420 ± 1774	17514 ± 6504	2726 ± 938	3748 ± 1253	3204 ± 999
360	31	61	883	215	1516	411	294	1196
360	32	102	1073	263	1859	801	379	381
360	33	105	982	324	2451	458	152	650
360	34	104	1125	172	3637	474	168	1034
360	35	105	1238	160	1374	402	172	N.D.
360	36	78	828	204	1658	692	300	N.D.
360	37	82	1276	320	2492	519	486	467
360	38	63	646	211	1955	436	264	283
360	39	51	840	346	1960	516	180	262
360	40	76	928	217	2667	500	136	714
Mean value (±S.D.)		83 ± 19	982 ± 187	243 ± 63	2157 ± 639	522 ± 122	253 ± 103	496 ± 359

gously as it was confirmed in our previous experimental study with another structurally related hallucinogenic drug, DOB in rats (Berankova et al., 2007). The lipophilicity of the parent drug carries the responsibility for its transport and retention in all tissues with impact on accumulation and elimination. However, taking into account the electron withdrawing effects of bromine substituent at the para position of the phenyl ring, having influence on the ionic form of the molecule, we cannot exclude that several mechanisms contribute to the influx and following accumulation of 2C-B in tissues, e.g. active transport in addition to passive diffusion.

Our results demonstrate also the significant 2C-B drug retention in the lung. We have found very high concentration levels of 2C-B in the lung and these experimental findings were in accordance with previous reports on the more potent hallucinogen and structurally related substance DOB, differing only in the alkyl counterpart of the molecule (Berankova et al., 2007). The DOB accumulation in the lung was mentioned previously

by Sargent et al. (1975) and by Shulgin and Shulgin (1991) with no mention about this phenomenon concerned 2C-B.

2C-B and some structurally related compounds, as mescaline or DOB, have been studied on biotransformation with various animal models (e.g. Scheline, 1978; Carmo et al., 2004, 2005; Kanamori et al., 2002, 2003, 2005; de Boer et al., 1998, 1999b; Theobald et al., 2007; Berankova and Balikova, 2005; Berankova et al., 2007; Ewald et al., 2007). Summing up, two metabolic pathways have been considered in the literature mentioned above, *O*-demethylation and oxidative deamination using different enzyme systems. Nevertheless the temporal disposition of metabolites either their psychoactivities have not been studied yet. In our experiments, the *O*-demethylated metabolite 2H5M-BPEA was determined in rat serum and tissues as the prevailing biodegradation product. The abundance of the other *O*-demethylated metabolite 2M5H-BPEA was negligible in all studied samples and its quantitative evaluation could not be performed. Our experimental findings correspond to data published by Kanamori et al. (2005) who found 2H5M-BPEA as the major metabolite in isolated rat hepatocytes additionally to the other metabolite 4-bromo-2,5-dimethoxyphenylacetic acid. The abundance of both metabolites was dose and time dependent; after higher dose the 2H5M-BPEA was prevailing above the acidic metabolite. The presence of this latter metabolite, 4-bromo-2,5-dimethoxyphenylacetic acid, was also indicated in our rat samples analyzed by GC–MS considering fragmentation mechanisms in mass spectra; however, it cannot be confirmed fully and quantified as the appropriate reference standard was not available.

The experimental data enabled us to estimate the basic kinetic parameters of 2C-B in rats. From the linear semilogarithmic plot of serum concentration vs. time (Fig. 5) the elimination half-life value ($t_{1/2}$) was approximately as 1.1 h, distribution volume (V_d) 16 L/kg and clearance (Cl) 9.8 L/h. These values are the first attempt to evaluate the pharmacokinetics of 2C-B under controlled conditions even if these preliminary results should be more verified by additional experiments. The validity of our data remains to be compared with results of other animal experiments, with various doses and administration ways, and to consider to what extent the animal data can be useful for assessment the human case of intoxication. Nevertheless, Carmo et al. (2005) observed minor interspecies differences in 2C-B metabolism and toxicity tests but large inter-individual differences in susceptibility of hepatocytes from three human donors.

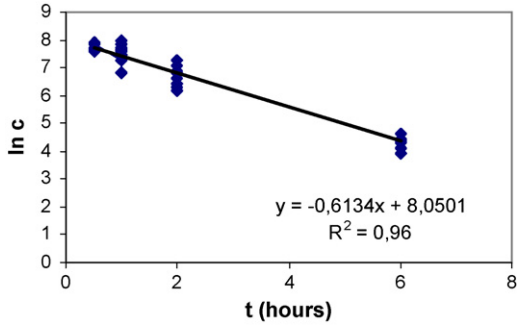

Fig. 5. Semilogarithmic plot of 2C-B serum concentration vs. time after application ($n = 10$).

In conclusion, the temporal profile of the disposition of the psychedelic 2C-B provides the first approximate estimation of kinetic parameters of 2C-B based on controlled animal experiment. The drug's ability to accumulate in the lung and persist in the brain after a higher dose can be important also from the perspective of subsequent behavioral study and interpretation.

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