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Distribution profile of 2,5-dimethoxy-4-bromoamphetamine (DOB) in rats after oral and subcutaneous doses

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Abstract

2,5-Dimethoxy-4-bromoamphetamine (DOB) is one of the potent hallucinogenic phenylalkylamines, whose ingestion has already caused several deaths reported all over the world. However, there is unsufficient information on DOB properties based on controlled pharmacokinetic studies available. The aim of this study was to clarify the distribution profile of DOB and its phenolic metabolite 2-methoxy-5-hydroxy-4-bromoamphetamine (2M5H4BA) in blood and biological tissues of experimental rats. The rats were administered a 20 mg/kg dose of DOB·HCl by oral ingestion or subcutaneous injection. Plasma and brain, liver and lung tissues were collected at 0.5, 1, 2, 4, 8, 16, and 32 h after dosing (three animals per time point). The samples were prepared by a liquid–liquid extraction procedure and the extracts were assayed by GC–MS. After per oral application, DOB peak plasma level of 320 ng/mL was reached after one-hour post dosing as well as 2M5H4BA peak concentration of 203 ng/mL. A rapid phase of DOB absorption, 2M5H4BA formation and their tissue distribution during the first two hours after application were followed by a slow decrease rate of the elimination process until 32 h. After subcutaneous application, high plasma levels of the unchanged parent drug and relatively reduced formation of its metabolite 2M5H4BA were observed. DOB maximum plasma concentration of 1143 ng/mL was reached after one-hour post application, whereas its metabolite peak level after 8 h was 213 ng/mL. The concentration profiles of both compounds in plasma after per oral and subcutaneous administration revealed the existence of significant first pass effect after per oral administration that significantly affected DOB bioavailability. DOB tissue concentrations exceeded plasma and the highest values were found in the lungs, where drug accumulation occurred with prolonged retention till 32 h after subcutaneous dose. Although the plasma/tissue transfer was more effective for the lipophilic parent drug than for its hydroxylated metabolite 2M5H4BA, the metabolite tissue levels were significant. The hallucinogenic potential of 2M5H4BA appearing in brain remains unclear as nothing is known about its pharmacological activity at present.

Keywords: 2,5-Dimethoxy-4-bromoamphetamine; DOB; Metabolites; Disposition in rats; Toxicological analysis

1. Introduction

Amphetamines and some of their structural analogs are drugs of abuse and cause serious health and social problems in many countries. In addition to the classical stimulants such as amphetamine and methamphetamine, there are structurally modified phenylalkylamines widely abused as recreational drugs, also known as "designer drugs". Many of these phenylalkylamine related compounds display not only stimulating but also psychedelic properties [1]. Some of these illicit drugs, for example, 3,4-methylendioxymethamphetamine (MDMA) or so-called 2C-series of phenylalkylamine designer drugs, became very popular especially among youngsters in dance parties [2,3]. Information about the occurrence and consumption of some other derivatives on the black market is scarce and there is also a lack of data describing their properties. One of these less abundant drugs is 2,5-dimethoxy-4-bromoamphetamine (DOB, brolamphetamine).

DOB was first synthesized by Shulgin and Shulgin, who described the synthetic procedure and reported on the effects of this compound [1]. DOB psychoactive effect is mediated via $5HT_{2A/2C}$ receptors interaction in the central nervous system [4,5] and is similar to that exhibited by other hallucinogenic phenylalkylamines, for example, mescalin [6,7]. A clinical human study [8] found that 2 mg oral dose of DOB produced emotional stimulation, perceptual enhancement, but not

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perceptual distortions or hallucinations. However, uncontrolled illegal use of potential higher doses may lead to hallucinations, panic state, vasospasms, coma, and even death [1,9–11]. To this date, DOB has been known as one of the most potent hallucinogens in the series of phenylalkylamines. DOB may be available on the illicit market in forms of tablets or blotters [10,12], and sometimes it can be misrepresented as LSD [13].

It was reported that DOB distributes first and foremost to the human lungs where it accumulates before the level in the brain builds up and therefore the onset of the effects is slow. The duration of the effects is 18–30 h long [1]. Sargent et al. have also set up the hypothesis that some metabolic conversion occurs in the lungs and this active metabolite may be responsible for the central action [14].

More detailed scientific information on the disposition of DOB in mammals would be desirable even if the systematic clinical study with human volunteers remains difficult due to ethical restrictions. Previously, some structurally related compounds, as mescaline [15], have been subjected to biotransformation studies with various animal models. Two competitive metabolic pathways, N-oxidation and O-demethylation employing different enzyme systems have been considered. In the case report [11] on two poisoned persons with DOB, the significant occurence of O-demethylated metabolite in urine was proposed. Its conjugated form in urine was prevailing above the traces of the free one. In the additional study, [16] using GC–MS with three different derivatives and synthesized reference standards of both isomers of mono O-demethylated metabolites in positions 2 or 5 of the aromatic ring, it was shown that the 5-O-desmethylmetabolite of DOB (2-methoxy-5-hydroxy-4-bromoamphetamine) was the major metabolite in the samples of human urine. Recently, the results of the systematic study on DOB metabolism and toxicological detection in rat urine have been published [17]. The authors confirmed that DOB was metabolized even in rats by O-demethylation followed by oxidative deamination to the corresponding ketone followed by reduction to the corresponding alcohol via hydroxylation of the side chain. Taking into account the published data about the biotransformation pathways of DOB and qualitative comments on its distribution, the aims of the presented study is to clarify the kinetic profile of DOB and its phenolic metabolite in blood and biological tissues of experimental rats. The disposition data on DOB and its metabolites related to the time of administration and data on drug persistance in brain may be useful for explanation of observed neuropharmacological effects and assessment of a real toxicological case with respect to laboratory results.

2. Materials and methods

2.1. Chemicals

DOB·HCl and its phenolic metabolite 2-methoxy-5-hydroxy-4-bromamphetamine (2M5H4BA) were synthesized for our purpose (Pharmaceutical Faculty of Charles University, Hradec Králové, Czech Republic) with NMR structural verification and purity of 85% (DOB·HCl) and 70% (2M5H4BA·HCl). The synthesis of DOB·HCl was performed by the procedure described by Shulgin and Shulgin [1]. The 2M5H4BA·HCl was synthesized by the modified procedure originally described for 2C-B metabolites by Kanamori et al. [18]. The modification consisted in using nitroethane for condensation and shorter reaction times. The ^{1}H and ^{13}C NMR spectra of the final product indicated presence of 70% of 2M5H4BA·HCl and the rest accounted for the debrominated substance. Analytical reference standard DOB·HBr was provided by Sigma Aldrich Ltd. (Prague, Czech Republic). Deuterated internal standard methylenedioxymethamphetamine-D5 (MDMA-D5) was purchased from Lipomed AG (Arlesheim, Switzerland). All other chemicals and solvents were of analytical grade.

2.2. Drug administration and sampling

Male Wistar rats (Velaz Ltd, Prague, Czech Republic) weighting 0.250 kg were used for this study. All experiments were performed in compliance with the guidelines and laws governing animal studies in the Czech Republic. The animals were housed in a temperature-controlled room at 20 °C with a 12/12 h lighting schedule. Water and food were provided *ad libitum*. The animals were administered a 20 mg/kg bolus dose of DOB·HCl aqueous solution either by gastric intubation or subcutaneously and were sacrificed at 0.5, 1, 2, 4, 8, 16, and 32 h after dosing in order to obtain plasma and brain, lung and liver tissues. Three animals were available at each time point. Data that represent the concentration drug profile at 0 h time point were obtained from animals without any previous DOB treatment. The samples were stored at −20 °C without any preservative for up to three months until analyses.

2.3. Sample preparation

2.3.1. Plasma

One milliliter of plasma from each animal was diluted by adding the same volume of distilled water and mixed with 100 ng of internal standard MDMA-D5. The sample was then processed by liquid–liquid extraction procedure. An amount of 0.5 mL of 1 M NaOH and 4 mL of ethylacetate were added and vortexed for 5 min. After centrifugation (4000 × rpm), the organic layer was transferred into a clean test tube and extracted for further 5 min with 1 mL of 1 M HCl. The acid aqueous layer was kept and after setting its pH value to 12 (1 M NaOH) all analytes were back extracted into 4 mL of ethylacetate. Three milliliters of ethylacetate was transferred into a clean glass tube and acetylated at 60 °C for 30 min with acetanhydride/pyridine (10:1) derivatization mixture, then evaporated and reconstituted with 100 μL of anhydrous ethylacetate.

2.3.2. Tissues

One gram of brain, lung or liver tissues, 5 mL of methanol and 1000 ng of MDMA-D5 was homogenised with Ultra-Turrax T25 homogenizer (Ika® Werke, Germany) followed by sonification (20 min) and centrifugation (3 min at 4000 × rpm). Three milliliters of supernatant were transferred to a clean glass test-tube and were evaporated. The residue was reconstituted with 200 μL of 0.1 M phosphate buffer (pH 6). The following procedure of liquid–liquid extraction was identical with the one used for plasma sample preparation.

2.4. GC–MS conditions

GC–MS Hewlett-Packard instrument HP 6890–5973 (Agilent, Waldbronn, Germany) equipped with autosampler, splitless injector, capillary column HP5-MS 30 m × 0.25 mm × 0.25 μm were used for analysis. Carrier gas was helium at constant flow of 1 mL/min. GC temperature program was in the range of 85–250 °C. One microliter of acetylated extract was injected at 250 °C. Mass spectrometer was operating in SIM mode with standard EI ionization at 70 eV. Selective ions monitored for each analyte are listed in Table 1.

Table 1
GC–MS monitored ions for DOB, 2M5H4BA and MDMA-D5 in acetylated forms

DOB·AC	**317**, **315***, 258, 256, 231, 229, 201, 199, 162, 86
2M5H4BA·2AC	**345**, **343**, 303, 301, 286, 284, 244, 242*, 86, 44
MDMA-D5·AC	**240***, 164, 136, 104, 62

m/z: Molecular ion; *m/z: quantification ion

2.5. Method validation

Blank rat plasma, brain, liver, and lung tissues were used for method validation process and for calibration purposes. For quantification internal standard method was used.

The calibration curves were constructed using linear regression analysis based on the ratio of the peak area of DOB·HCl or 2M5H4BA·HCl to the peak area of the internal standard MDMA-D5. Two replicates were assayed for each calibration level. For brain and plasma a five-point calibration model was used, for liver and lung tissues a seven-point linearity was established.

3. Results and discussion

This study was designed to assess the influence of per oral and subcutaneous DOB administration on DOB and its main metabolite 2-methoxy-5-hydroxy-4-bromoamphetamine distribution profiles in rat tissues. The presence of 2M5H4BA as the most abundant DOB metabolite was proved in previous studies on DOB metabolism in humans using human urine from two intoxicated subjects [11,16] as well as in the rat (unpublished data). The identification and confirmation of 2M5H4BA in our samples was performed comparing a reference standard mass spectra and retention data. Typical mass spectra of both analytes are shown in Fig. 1 with a plasma extract chromatogram.

3.1. Method validation results

For plasma, the linearity was determined in the range of 5 to 2000 ng/mL with regression coefficient of 0.9995 for DOB and 0.9975 for 2M5H4BA. In the brain, the linearity was established to be within 20–20,000 ng/g range with DOB regression coefficient of 0.9960 and 0.9991 for 2M5H4BA. Liver and lung tissue calibration curves were found to be linear even between 50 and 90,000 ng/g. Coefficients of regression were as follows: 0.9954 for DOB and 0.9987 for 2M5H4BA.

LOD ($s/n > 3$) were lower than 1 ng/mL in plasma and 10 ng/g in other tissues. LLOQ ($s/n > 10$) were determined as follows: 5 ng/mL for DOB and 10 ng/mL for 2M5H4BA in plasma, 20 ng/g for both compounds in the brain and 50 ng/g for DOB and 2M5H4BA in liver and lung tissues.

Intra-assay precision ($n = 6$) reported as RSD was in all tested samples lower than or equal to $\pm$ 10% and was calculated for both analytes at the lowest (LLOQ), middle and highest level of the calibration range for each matrix. The investigated middle values corresponded with 500 and 1000 ng/mL for plasma, for organs the respective levels were 1000 and 10,000 ng/g for DOB and 2M5H4BA.

3.2. DOB and 2M5H4BA plasma concentrations after per oral and subcutaneous administration

Plasma concentration profiles of both DOB and 2M5H4BA are presented in Fig. 2. After per oral application, DOB peak level (320 ng/mL) was reached after one-hour post dosing as well as 2M5H4BA peak concentration of 203 ng/mL. It is evident that DOB biotransformation in the liver via first pass effect was fast and DOB bioavailability was therefore much reduced. A rapid phase of DOB absorption, 2M5H4BA formation and their tissue distribution during the first two hours after application were followed by a slow decrease rate of the elimination process until 32 h.

The data after subcutaneous administration confirm the importance of the liver in the first pass metabolism as discussed above. In the case of subcutaneous dose, high plasma levels of the unchanged parent drug and reduced and delayed formation

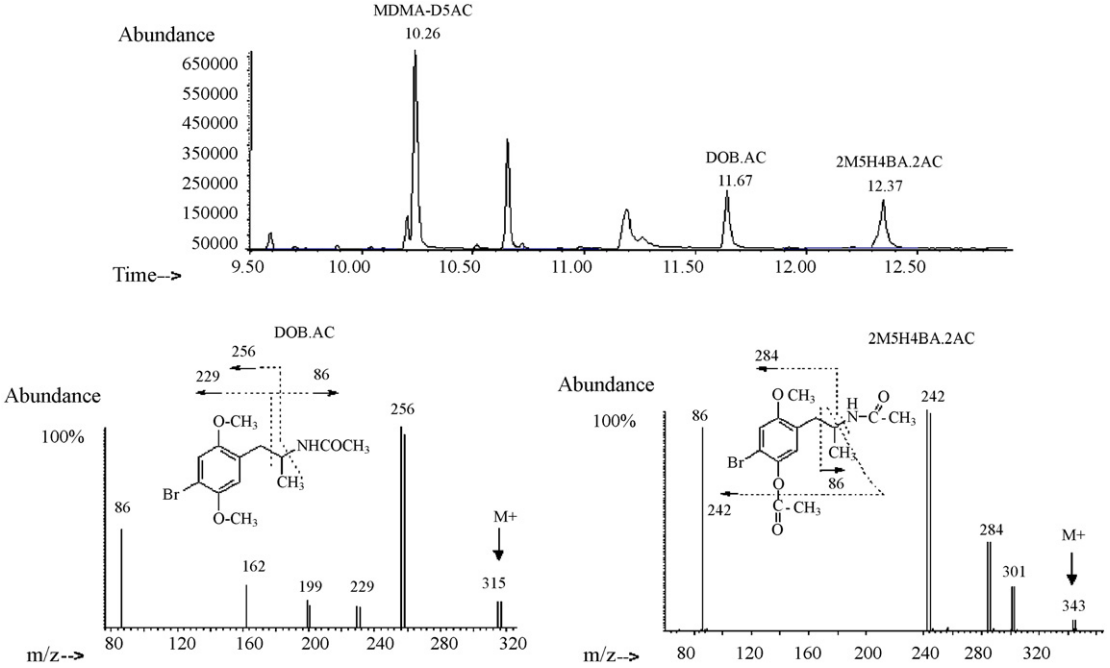

Fig. 1. Typical chromatogram of an acetylated plasma extract with DOB·AC, the phenolic metabolite 2M5H4BA·2AC and internal standard MDMA-D5·AC and SIM mass spectra of DOB·AC and 2M5H4BA·2AC.

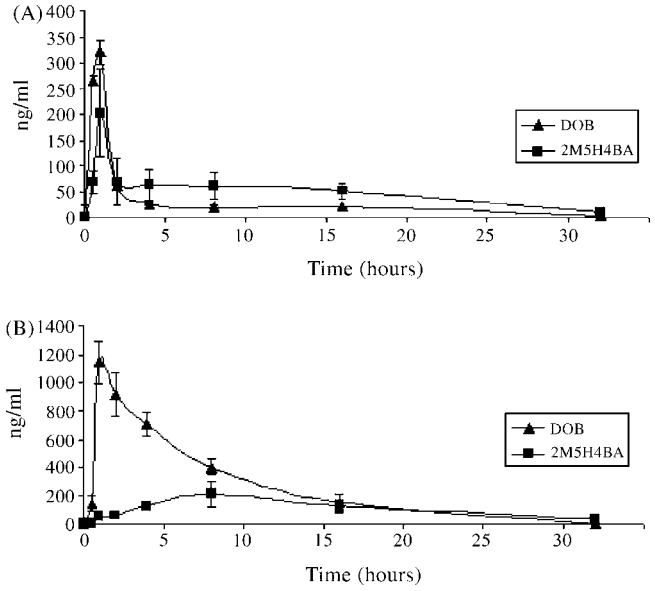

Fig. 2. Comparison between plasma concentration time course of DOB and its metabolite 2M5H4BA in rats after oral (A) and subcutaneous (B) administration of a 20 mg/kg DOB bolus dose. Results given as an average concentration ±S.D. ($n = 3$ animals per time point).

of 2M5H4BA were observed. DOB top level (1143 ng/mL) was reached after one-hour post dosing, whereas its metabolite peak level after 8 h with a value of 213 ng/mL only. DOB fast absorption into plasma was similar to that after per oral application, nevertheless the following decrease was more gradual. Based on our experimental data, we estimated DOB elimination half-time in the rat to be 5 h approximately as follows from the semilogaritmic concentration versus time plot after subcutaneous dose. This half-time value should be considered as preliminary only due to the limited number of animals and should be verified further.

3.3. DOB and 2M5H4BA distribution in tissues

As DOB is a potent psychedelic and also lipophilic substance, high concentrations were expected in the brain. Fig. 3 presents DOB brain and plasma amounts after per oral and subcutaneous administration. These data indicate that DOB influx into the brain was significant, with strong accumulation especially after subcutaneous dose, which is essential for DOB hallucinogenic and long-lasting effects.

Both plasma and brain per oral curves were characterized with rapid increase, in the brain up to 7600 ng/g, and fast elimination. At 32 h after the dose, DOB amount was under LLOQ in plasma and the brain as well. The maximum brain/plasma ratio was reached in 2 h after the dose and showed only a short delay after the peak concentrations in both tissues.

DOB plasma (1143 ng/mL) and brain (14,157 ng/g) peak concentrations after subcutaneous dose were several times higher than those after per oral dose due to the parenteral application with high bioavailability. DOB influx into the brain was slower, which was characterized by one hour delayed peak concentration after the plasma maximum was reached. High brain concentrations were still observed after 16 h or even 32 h and the maximum brain/plasma ratio appeared no sooner than 8 h post dosing.

Fig. 4 shows DOB distribution profile in brain, liver, and lung tissues. After per oral dose, the concentration maximum in all tissues was reached 1 h post application. The highest level was observed in the lungs (25,318 ng/g), then in the brain (7605 ng/g), and liver (7276 ng/g). All the tissues exhibited fast DOB absorption and gradual exponential elimination. After a

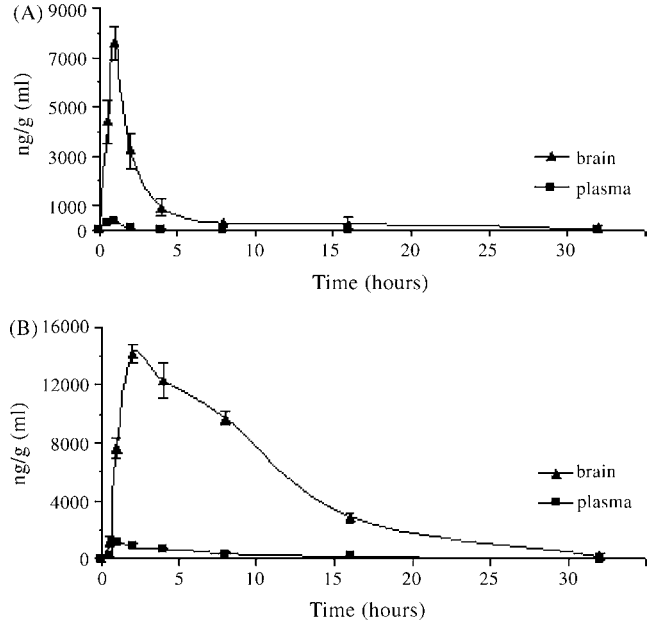

Fig. 3. DOB brain and plasma time course in rats after per oral (A) and subcutaneous (B) administration of a 20 mg/kg bolus dose. Results given as an average concentration ±S.D. ($n = 3$ animals per time point).

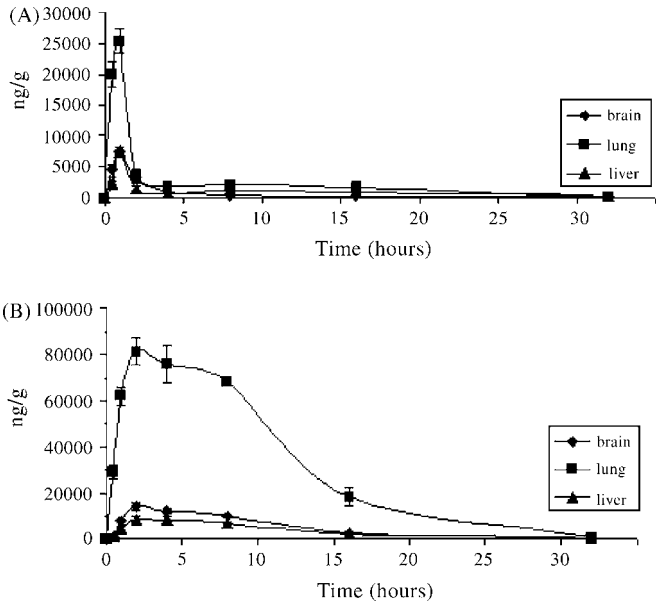

Fig. 4. DOB time profile in rat brain, lung, and liver tissues after oral (A) and subcutaneous (B) administration of a 20 mg/kg DOB bolus dose. Results given as an average concentration ±S.D. ($n = 3$ animals per time point).

Table 2
2M5H4BA rat tissue concentration time profile after per oral DOB administration (20 mg/kg)

Tissue	2M5H4BA concentration (ng/g) ±S.D.						
	0.5 h	1 h	2 h	4 h	8 h	16 h	32 h
Brain	410 ± 314	624 ± 312	3754 ± 3473	NR	1109 ± 835	1125 ± 1113	75 ± 107
Lung	1494 ± 403	2070 ± 187	808 ± 564	357 ± 114	1953 ± 212	1334 ± 909	81 ± 9
Liver	1847 ± 652	4467 ± 378	3017 ± 709	2010 ± 331	2612 ± 169	1272 ± 547	371 ± 351

2M5H4BA: 2-methoxy-5-hydroxy-4-bromoamphetamine; S.D.: standard deviation; NR: not reported (below LLOQ); results given as an average concentration ($n = 3$ animals per time point).

Table 3
2M5H4BA rat tissue concentration time profile after subcutaneous DOB administration (20 mg/kg)

Tissue	2M5H4BA concentration (ng/g) ±S.D.						
	0.5 h	1 h	2 h	4 h	8 h	16 h	32 h
Brain	NR	NR	1375 ± 387	2896 ± 129	5725 ± 566	3440 ± 267	187 ± 136
Lung	391 ± 8	1219 ± 885	2739 ± 624	4875 ± 832	18208 ± 693	10322 ± 2030	339 ± 32
Liver	648 ± 21	2864 ± 698	5000 ± 1089	9483 ± 2190	5015 ± 196	2304 ± 1178	200 ± 60

2M5H4BA: 2-methoxy-5-hydroxy-4-bromoamphetamine; S.D.: standard deviation; NR: not reported (below LLOQ); results given as an average concentration ($n = 3$ animals per time point).

subcutaneous dose with high bioavailability, the distribution phase was prolonged and the concentration maximum in brain (14,157 ng/g) and liver (8473 ng/g) were ascertained 2 h after dosing. In lungs, the DOB level was extremely high and the maximum value of 81,216 ng/g was found. Significant amounts of DOB were still observed after 32 h: 219 ng/g in the brain, 149 ng/g in the liver, and 441 ng/g in the lungs. The lipophilicity of the parent drug is responsible for its uptake and retention in tissues with impacts on accumulation and slow elimination. The extreme and retained DOB levels found in lungs were surprising, nevertheless confirming the previous reports [1,14]. The published results after intravenous application of DOB to humans [14] also demonstrated fast DOB appearance in lungs, while the influx into the liver and brain was delayed. The authors [14] suggested the potential existence of an active DOB metabolite, which was formed either in the lungs or liver before its appearance in the brain.

In our study, 2M5H4BA levels exhibited great individual variability in all tissues, especially in brain, reflecting the metabolic variation in animals. The experimental data are summarized in Tables 2 and 3. In general, the maximum 2M5H4BA levels in lungs and brain were much lower than those reported for the parent drug. In brain, the levels of the metabolite reached the parent drug values with a delay of 2 h after per oral dose and 16 h after subcutaneous dose approximately. In liver, the DOB and 2M5H4BA levels were very close. However, this could be expected because liver plays the most important role in DOB metabolism. Since 2M5H4BA was detected all over the body, its occurence in organs, especially in brain, might be important from pharmacodynamic perspective. However, to date neither 2M5H4BA nor any other metabolite of DOB has been identified as active.

4. Conclusions

In this study, DOB and its metabolite 2-methoxy-5-hydroxy-4-bromoamphetamine distribution time profiles in the rat after per oral and subcutaneous dose were compared. This is the first controlled pharmacokinetic study with this drug to our knowledge. It can be concluded that DOB was extensively metabolised via demethylation step to its metabolite 2M5H4BA in the liver. The importance of the liver in DOB biotransformation was confirmed and the bioavailability of the drug after per oral dose was reduced due to the existence of the first pass effect. The DOB high brain/plasma ratio together with its slow release from tissues can explain its strong and prolonged hallucinogenic potential. Also in other tissues, the parent drug was found in high levels, especially in the lungs. In this lung tissue, masive accumulation with slow release was ascertained. As expected, in all tissues the plasma/organ transfer was more effective for the lipophilic parent drug than for its hydroxylated metabolite 2M5H4BA. However, the metabolite concentration was still significant in all investigated tissues. The pharmacological activity of 2M5H4BA has not yet been investigated; therefore, it remains to be elucidated in further studies whether this metabolite might play an important role in DOB long-term effects.

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