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EXCRETION OF METHOXYPHENAMINE AND ITS METABOLITES IN RAT URINE

TETSUKICHI NIWAGUCHI and TAKADO INOUE
National Research Institute of Police Science, 6, Sanban-cho, Chiyoda-ku, Tokyo (Japan)
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SUMMARY

Metabolites of methoxyphenamine in urine obtained after oral administration of the drug to rats have been isolated by Sephadex LH-20 column chromatography and identified as the O-, N,O- and N-demethylated derivatives of methoxyphenamine by thin-layer chromatography, spectrometry, and comparisons with synthesized compounds. The unchanged drug and the metabolites excreted in urine have been extracted and determined by gas chromatography after treatment with trifluoroacetic anhydride.

INTRODUCTION

It was shown by Chundela and Šlechtová[1] that one of the metabolites of methoxyphenamine in humans is the N-demethylated derivative, 2-amino-1-(*o*-methoxyphenyl)propane, and another metabolite may be either N-hydroxylated methoxyphenamine or 1-(*o*-methoxyphenyl)-2-propanol. Later, Midha and Coutts[2] demonstrated that the mass spectrum of another metabolite found in the previous study was clearly different from those of synthesized N-hydroxylated methoxyphenamine and 1-(*o*-methoxyphenyl)-2-propanol, but it was identical to that of the O-demethylated derivative, 1-(*o*-hydroxyphenyl)-2-methylaminopropane.

In this study, the metabolites of methoxyphenamine found in urine after oral administration of the compound to rats have been isolated and identified by comparing their analytical data with those of synthesized 2-amino-1-(*o*-methoxyphenyl)-propane, 1-(*o*-hydroxyphenyl)-2-methylaminopropane and 2-amino-1-(*o*-hydroxyphenyl)propane. The main metabolic route of methoxyphenamine in rat is O-demethylation and the major metabolite is 1-(*o*-hydroxyphenyl)-2-methylaminopropane together with small amounts of 2-amino-1-(*o*-hydroxyphenyl)propane and 2-amino-1-(*o*-methoxyphenyl)propane.

EXPERIMENTAL

Materials

Methoxyphenamine hydrochloride was extracted with chloroform from Orthoxin (Japan Upjohn, Tokyo, Japan). The extract was recrystallized from ethanol–diethyl ether (1:1) to give the required product, m.p. 130–132°.

1-(o-Hydroxyphenyl)-2-methylaminopropane hydrochloride. A mixture of 1 g of methoxyphenamine and 6 ml of 48% hydrobromic acid was refluxed for 2 h. The reaction mixture was made alkaline, with sodium carbonate and then extracted with diethyl ether. Hydrogen chloride was passed into the ether extract and the ether was evaporated. The residue obtained was recrystallized from ethanol–diethyl ether (1:1) to give the required product, m.p. 165–166°.

2-Amino-1-(o-methoxyphenyl)propane hydrochloride. To a mixture of 5 ml of tetrahydrofuran and 3.5 g of lithium aluminum hydride, was added dropwise 5 ml of a tetrahydrofuran solution containing 5 g of 1-(*o*-methoxyphenyl)-2-nitro-1-propene which was synthesized by the method of Heinzelman[3]. The mixture was refluxed for 4 h, then filtered and the filtrate was evaporated under reduced pressure. The residue was dissolved in diethyl ether and extracted with 1 *N* hydrochloric acid. The aqueous layer was separated, made alkaline with sodium carbonate and extracted with diethyl ether. Hydrogen chloride was passed into the ether solution and the ether was evaporated. The residue was recrystallized from ethanol–diethyl ether (1:1) to give the required product, m.p. 111–112°.

2-Amino-1-(o-hydroxyphenyl)propane hydrochloride. 2-Amino-1-(*o*-methoxyphenyl)propane was demethylated at a methoxyl radical in the same manner as in the synthesis of 1-(*o*-hydroxyphenyl)-2-methylaminopropane. The product had m.p. 160–161°.

Sephadex LH-20 and β-glucuronidase were purchased from Pharmacia (Uppsala, Sweden) and Sigma (St. Louis, Mo., U.S.A.), respectively, and all of the other chemicals were special grade.

Isolation of metabolites

Methoxyphenamine hydrochloride (50 mg/kg) was given orally as 10 mg/ml aqueous solution to five male Wistar rats weighing 150–200 g. The animals were placed in individual cages and their urine was collected every 24 h.

The urine was adjusted to pH 9.0 with sodium carbonate and extracted four times with equal volumes of chloroform–isopropanol (3:1). The solvent was evaporated under reduced pressure, after drying over anhydrous sodium sulphate and adding a drop of acetic acid to prevent evaporation of the compounds. The unchanged drug and the metabolites were contained in the residue. The urine remaining from the extraction was neutralized with hydrochloric acid. A volume of concentrated hydrochloric acid equal to one fifth of the solution volume was added and the mixture was heated on a boiling water-bath for 1 h in order to hydrolyze conjugated metabolites. The resulting solution was neutralized with sodium hydroxide and adjusted to pH 9.0 with sodium carbonate, followed by an extraction with chloroform–isopropanol as described above.

For the identification of the metabolites a large-scale experiment was carried out as follows. Urine, which had been collected for 24 h after oral administration of methoxyphenamine to 100 male rats, was extracted with chloroform–isopropanol (3:1) as described above. The extract was dissolved in a small volume of methanol and separated by column chromatography on Sephadex LH-20 into four fractions, eluted with acetone (fractions A and B), with acetone–methanol (98:2) (fraction C) and with acetone–methanol (95:5) (fraction D).

Analytical procedures

Thin-layer chromatography (TLC). TLC was carried out on 250-μm layers of silica gel GF_{254} (E. Merck, Darmstadt, G.F.R.). The solvent systems used for development were chloroform saturated with 28% ammonium hydroxide–ethanol–methanol (18:1:1) (A), methanol–28% ammonium hydroxide (100:1.5) (B) and chloroform–methanol–acetic acid (15:4:1) (C). Chromatograms were visualized under an ultraviolet (UV) lamp (254 nm) and by spraying the following reagents: 20% sodium carbonate solution, 1% sodium nitroprusside solution and 50% acetaldehyde in ethanol (I); 0.1% 2,6-dichloroquinone-4-chloroimide in ethanol and ammonia (II); or 0.25% fluorescamine in acetone and 10% triethylamine in methylene chloride (III).

Gas chromatography (GC). A Shimadzu GC-4CM gas chromatograph equipped with a flame ionization detector was used. The glass column (2 m × 3 mm I.D.) was packed with 2% OV-17 on Chromosorb W AW DMCS (80–100 mesh) as the stationary phase. Temperatures: oven, 130°; injection port, 180°; detector, 180°. Nitrogen flow-rate, 50 ml/min. The chloroform–isopropanol extract obtained from the urine was dissolved in 200 μl of ethyl acetate and 200 μl of trifluoroacetic anhydride were added. The vessel containing the mixture was stoppered tightly and heated at 55° for 20 min. The solvent was evaporated under reduced pressure and the residue obtained was dissolved in ethyl acetate; 1 μl of this solution was injected into the gas chromatograph. For the determination of the unchanged drug and the metabolites, 0.15 μg/μl of bibenzyl in ethyl acetate was used as an internal standard. A Shimadzu Chromatopac-1A digital-integration computing system was used for integration and peak identification.

UV, infrared (IR) and mass spectra were obtained with Shimadzu MPS-5000, JASCO DS-701G and JEOL OIS-G instruments, respectively.

RESULTS AND DISCUSSION

Identification of the metabolites

Four spots were recognized on thin-layer chromatograms of the chloroform–isopropanol extract of urine obtained after administration of methoxyphenamine (Table I). One of the four spots, which gave a blue colour with reagent I, was identical to the unchanged drug, and the other three spots (M_1, M_2 and M_3) were considered to be metabolites of methoxyphenamine. It was presumed that the metabolites M_1, M_2 and M_3 were O-, N,O- and N-demethylated derivatives of methoxyphenamine, respectively, from the colours of the spots obtained by spraying with the various the reagents and from a comparison of the R_F values with those of synthesized 1-(*o*-hydroxyphenyl)-2-methylaminopropane, 2-amino-1-(*o*-hydroxyphenyl)propane and 2-amino-1-(*o*-methoxyphenyl)propane on thin-layer chromatograms.

Gas chromatographic analysis of the extract treated with trifluoroacetic anhydride showed three peaks other than that of the unchanged drug. The retention times from the gas chromatograms are compared with those of the synthesized compounds in Fig. 1.

Fraction A, obtained by Sephadex LH-20 column chromatography of the urine extract from the large-scale experiment, gave the metabolite M_1, corresponding to the spot having R_F 0.33 in solvent system A in TLC; fraction B, the unchanged drug, corresponded to the spot having R_F 0.48, fraction C, the metabolite M_3, corre-

TABLE I

R_F VALUES AND COLOUR REACTIONS OF METHOXYPHENAMINE AND ITS METABOLITES EXCRETED IN URINE

Solvent systems used for development were chloroform saturated with 28% ammonium hydroxide–ethanol–methanol (18:1:1) (A), methanol–28% ammonium hydroxide (100:1.5) (B) and chloroform–methanol–acetic acid (15:4:1) (C). Reagents used for colour development were 20% sodium carbonate solution, 1% sodium nitroprusside solution and 50% acetaldehyde in ethanol (I), 0.1% 2,6-dichloroquinone-4-chloroimide in ethanol and ammonia (II) and 0.25% fluorescamine in acetone and 10% triethylamine in methylene chloride (III).

Compound	*R_F value*			*Colour development*		
	Solvent system			*Reagent*		
	A	*B*	*C*	*I*	*II*	*III*
Unchanged methoxyphenamine	0.48	0.37	0.44	Blue	—	—
M_1	0.33	0.51	0.42	Blue	Light blue	—
M_2	0.27	0.53	0.33	—	Purple	Yellow fluor.*
M_3	0.43	0.48	0.50	—	—	Yellow fluor.*

* Yellow fluorescence under a UV lamp (365 nm) after spraying with reagent III.

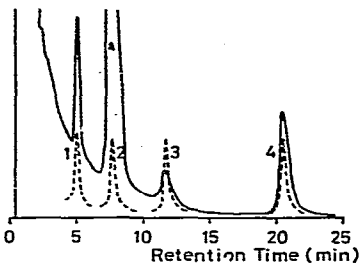

Fig. 1. Gas chromatograms of methoxyphenamine and its metabolites treated with trifluoroacetic anhydride. The solid line shows the trifluoroacetylated extract of urine obtained after oral administration of methoxyphenamine to rat. The dashed lines show authentic 2-amino-1-(*o*-hydroxyphenyl)propane (1), 1-(*o*-hydroxyphenyl)-2-methylaminopropane (2), 2-amino-1-(*o*-methoxyphenyl)propane (3) and methoxyphenamine (4).

sponded to the spot having R_F 0.43 and fraction D, the metabolite M_2, corresponded to the spot having R_F 0.27. The unchanged drug and the metabolites M_1, M_2 and M_3 separated by the column chromatography were recrystallized from ethanol–diethyl ether (1:1, v/v) as their hydrochlorides.

UV absorption spectra of the hydrochlorides in methanol are shown in Table II. The fact that the maximum absorptions of M_1 and M_2 in methanolic 1% sodium hydroxide solution are shifted by 1.5 nm to longer wavelengths than in methanol suggests that these compounds, possess phenolic OH groups.

Mass, high-resolution mass and IR spectra are shown in Fig. 2, Table III and Fig. 3, respectively. The analytical data of M_1, M_2 and M_3 are in excellent agreement with those of synthesized 1-(*o*-hydroxyphenyl)-2-methylaminopropane, 2-amino-1-(*o*-hydroxyphenyl)propane and 2-amino-1-(*o*-methoxyphenyl)propane, respectively. From these results it is concluded that M_1, M_2 and M_3 are the O-, N,O- and N-demethylated derivatives of methoxyphenamine, respectively (Fig. 4).

TABLE II

ULTRAVIOLET ABSORPTION OF METHOXYPHENAMINE AND ITS METABOLITES

Compound	$\lambda_{max.}$ *(nm)*			
	In methanol		*In 1% NaOH*	
Unchanged methoxyphenamine	273.0	279.0*	273.5	279.5*
M_1	275.5	280.5*	277.0*	282.0
M_2	276.0	281.5*	277.5*	283.0
M_3	273.0	279.0*	273.0	279.0*

* Shoulder.

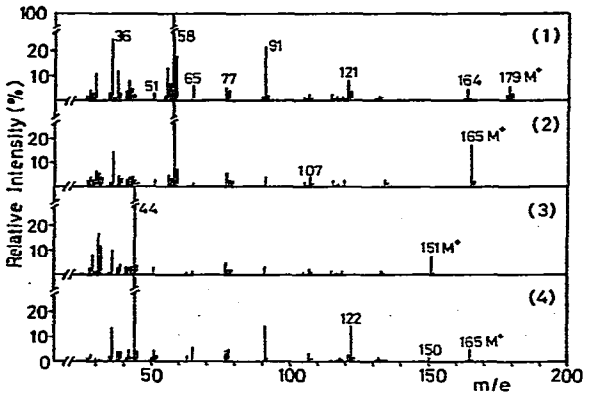

Fig. 2. Mass spectra of methoxyphenamine (1) and its metabolites M_1 (2), M_2 (3) and M_3 (4) which are isolated from the urine.

TABLE III

MASS MEASUREMENT FOR M^+ OF METHOXYPHENAMINE AND ITS METABOLITES BY HIGH-RESOLUTION MASS SPECTROMETRY

Compound	M^+		
	Found	*Calc.*	*Formula*
Unchanged methoxyphenamine	179.130	179.131	$C_{11}H_{17}NO$
M_1	165.114	165.115	$C_{10}H_{15}NO$
M_2	151.100	151.100	$C_9H_{13}NO$
M_3	165.117	165.115	$C_{10}H_{15}NO$

Excretion of the unchanged drug and the metabolites in rat urine

Recovery of methoxyphenamine, 2-amino-1-(*o*-methoxyphenyl)propane, 1-(*o*-hydroxyphenyl)-2-methylaminopropane and 2-amino-1-(*o*-hydroxyphenyl)propane, which were added to the urine of a control rat, was examined by GC after the extraction. When diethyl ether or chloroform was used as extraction solvent, 90–100% of the first three compounds was recovered, but only 70–80% of 2-amino-1-(*o*-hydroxyphenyl)propane. For the extraction the most suitable solvent was chloroform–isopropanol (3:1), the recovery of all the compounds ranging from 96 to 100%.

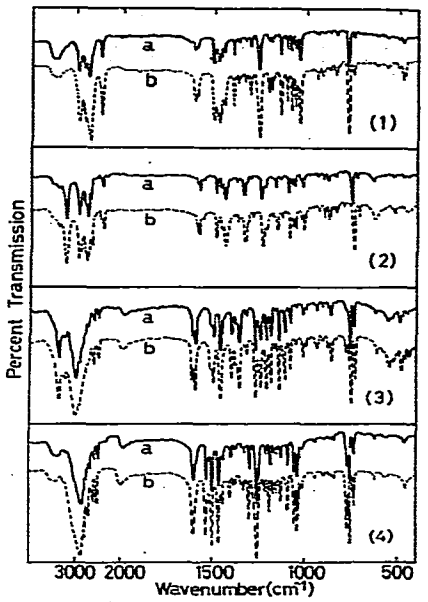

Fig. 3. Infra-red spectra of the hydrochlorides of methoxyphenamine and its metabolites in sodium chloride disks. The solid lines (a) show the hydrochlorides of methoxyphenamine (1), M_1 (2), M_2 (3) and M_3 (4) which are isolated from the urine. The dashed lines (b) show the hydrochlorides of authentic methoxyphenamine (1), 1-(*o*-hydroxyphenyl)-2-methylaminopropane (2), 2-amino-1-(*o*-hydroxyphenyl)propane (3) and 2-amino-1-(*o*-methoxyphenyl)propane (4).

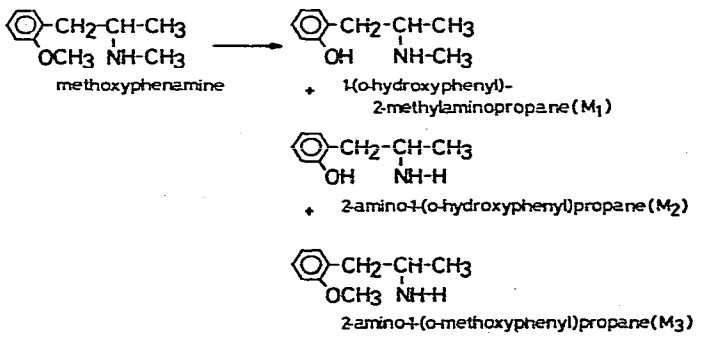

Fig. 4. The metabolites of methoxyphenamine excreted in rat urine.

In order to identify and determine conjugated metabolites, 10 ml of urine was adjusted to pH 5.0 with 10 ml of 0.2 *M* acetate buffer (pH 5.0) and acetic acid. *β*-Glucuronidase (3000 units) was then added and the mixture was incubated at 37° for 48 h. The incubation mixture was adjusted to pH 9.0 with sodium carbonate and extracted with chloroform–isopropanol (3:1). It was confirmed by TLC that the unchanged drug and the metabolites M_1, M_2 and M_3 were contained in the extract. The amounts of the metabolites in the extract were determined by GC, and compared with those in the urine before hydrolysis. It was shown that glucuronides of the

metabolites were not present in the urine, since the contents of the metabolites before and after hydrolysis were approximately equal. Hydrolysis with hydrochloric acid was attempted as follows. Concentrated hydrochloric acid was added to the urine to give a final acid concentration of 2 *N*. This mixture was then heated for various times from 0.5 to 2.5 h. Under all conditions of hydrolysis the metabolites M_1 and M_2 were identified by TLC. The maximum amounts of the metabolites were obtained after heating for 1 h and these amounts were unaltered by more longer periods of heating. This result suggests that conjugated metabolites other than glucuronides are excreted in urine. The amounts of unchanged drug and the metabolites in urine after oral administration of the drug to rats are shown in Table IV.

TABLE IV

URINARY EXCRETION OF METHOXYPHENAMINE AND ITS METABOLITES AFTER ORAL ADMINISTRATION TO RAT

n.d. = Not detected.

Metabolite	*Percentage of dose excreted*				
	Day after oral administration				
	1	*2*	*3*	*4*	*5*
Unchanged methoxyphenamine	3.8 ± 1.2	0.2 ± 0.2	n.d.		
1-(*o*-Hydroxyphenyl)-2-methlaminopropane, M_1					
free	14.8 ± 2.1	0.9 ± 0.7	0.3 ± 0.3	0.1 ± 0.1	trace
conjugated	1.7 ± 0.6	0.1 ± 0.1	trace	n.d.	
2-Amino-1-(*o*-hydroxyphenyl)propane, M_2					
free	2.8 ± 0.8	0.1 ± 0.1	trace	n.d.	
conjugated	0.7 ± 0.5	0.1 ± 0.1	n.d.		
2-Amino-1-(*o*-methoxyphenyl)propane, M_3	0.6 ± 0.2	0.1 ± 0.1	n.d.		

The unchanged drug and the metabolites excreted in rat urine comprised 26% of the dose when determined 4 days after oral administration, and the main metabolites were O-demethylated methoxyphenamine (17.9%) and N,O-demethylated methoxyphenamine (3.7%). These phenols were excreted mainly as the free compounds. Only 4.0% of the dose was excreted unchanged and 0.7% appeared as N-demethylated methoxyphenamine.

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