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SIMULTANEOUS ANALYSIS OF PHENYLGLYCOLS AND PHENYLETHANOLS IN HUMAN URINE BY GAS CHROMATOGRAPHY—MASS SPECTROMETRY

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SUMMARY

A method is described for the determination of the neutral metabolites formed from catecholamines and various other structurally related phenylethylamines by using gas chromatography—chemical ionization—mass spectrometry. These metabolites (phenylglycols and phenylethanols) were extracted from urine specimens and converted to pentafluoropropionyl derivatives which were separated on either 3% OV-1, 3% SP-2250, or 3% QF-1 packed columns. Our results demonstrate the presence in human urine of *p*-hydroxyphenylglycol, a metabolite of octopamine. One patient excreted 13 and 91 μg/day of free and total (free + conjugated) *p*-hydroxyphenylglycol, respectively. Treatment with a monoamine oxidase inhibitor reduced the excretion of total *p*-hydroxyphenylglycol to 30% of baseline level.

INTRODUCTION

The principal catabolic pathway of catecholamines, their O-methylated metabolites, and related phenylethylamines in neural tissues is via the oxidative deamination reaction catalyzed by monoamine oxidase. The aldehyde intermediates thus formed are metabolized further either by oxidation to a carboxylic acid or by reduction to an alcohol. Schanberg et al. [1] showed that 3-methoxy-4-hydroxyphenylethylene glycol (MHPG) is the major metabolite of norepinephrine in brain. Since norepinephrine is primarily converted in peripheral tissues to the acidic metabolite, vanilylmandelic acid (VMA), the urinary excretion of MHPG is often used as an index of norepinephrine metabolism in the central nervous system (CNS). The amount of MHPG excreted

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per day as the free glycol plus its conjugates is relatively large (about 1—2 mg in man) and can be readily determined by gas chromatography with electron-capture detection of the trifluoroacetyl [2,3] or pentafluoropropionyl (PFP) [4] derivatives. Other neutral metabolites are generally present in biological fluids in much lower concentrations, but their measurement may nevertheless be an important step in studying the metabolism of their parent amines. Several investigators [4—7] have used gas chromatographic methods combined with electron-capture detection or mass spectrometry to determine the levels of 3-methoxy-4-hydroxyphenylethanol (MHPE), a neutral metabolite of dopamine, in brain, cerebrospinal fluid and urine. Although there are some discrepancies among the levels reported by different laboratories, it is clear that MHPE is a minor metabolite of dopamine and the significance of measuring this metabolite is not yet known. On the other hand, we have shown that phenylethylene glycol (PEG) is the major deaminated metabolite of phenylethanolamine [8]. By using gas chromatography—mass spectrometry (GC—MS) we have been able to measure PEG in rat and human urine and we have found that its excretion is elevated in untreated phenylketonuria [9]. In this paper we describe a method using GC—MS which permits the simultaneous determination of PEG, *p*-hydroxyphenylethanol (pHPE), *p*-hydroxyphenylglycol (pHPG), and MHPE, which are neutral metabolites of phenylethanolamine, *p*-tyramine, octopamine and dopamine, respectively, and MHPG and dihydroxyphenylethylene glycol (DHPG), both metabolites of norepinephrine.

EXPERIMENTAL

Urine specimens were obtained from patients on the Clinical Research Unit at the Western Psychiatric Institute and Clinic. All patients were drug-free for at least two weeks. The urines were collected in containers to which sodium metabisulfite had been added as a preservative. The urines were kept cold during the 24-h collection period, after which they were frozen until assayed.

Urine samples (3 ml) were added to 1 ml of 1 *M* sodium acetate (pH 6.0) and 0.5 ml of 2% EDTA and treated with 0.1 ml glusulase (Endo Labs., Garden City, N.Y., U.S.A.) at 37° for 20 h as described by Dekirmenjian and Maas [3]. The samples were then extracted according to the detailed procedures given elsewhere [9]. Briefly, the neutral metabolites were extracted with 3 × 10 ml of ethyl acetate. The combined organic phase was washed with 1 ml of 1 *M* $KHCO_3$ and evaporated just to dryness at 43° in a rotary evaporator. The residue was dissolved in 2 ml of ethyl acetate and stored at 4°. Calibration curves were prepared by the addition of 0—120 μl of a standard mixture (containing 2.5 μg/ml of PEG, pHPE, pHPG and 100 μg/ml of MHPG) to separate aliquots of a pooled urine sample and carrying them through the extraction procedure, without the addition of glusulase.

Aliquots of 200 μl of the ethyl acetate extracts obtained from samples and standards were reacted for at least 15 min at room temperature with 25 μl of pentafluoropropionic anhydride (PFPA; Pierce, Rockford, Ill., U.S.A.). In order to completely derivatize *o*-hydroxyphenylethanol (oHPE), a somewhat longer reaction time (30 min) was required, presumably due to steric hindrance. Two microliters of the reaction mixture were then injected into a

Finnigan Model 3200F quadrupole gas chromatograph—mass spectrometer which was operated in the chemical ionization (CI) mode (electron energy 90 eV). The chromatographic separations were carried out on a 5 ft. × 2 mm I.D. silanized U-column packed with either 3% OV-1, 3% SP-2250, or 3% QF-1 on 80—100 mesh Supelcoport (Supelco, Bellefonte, Pa., U.S.A.) using temperature programming. The injection port was maintained at 235°. Methane, which served as both the carrier gas and the reagent gas, was adjusted to a flow-rate (about 10 ml/min) which gave an ion source pressure of 1.0 torr. At the same time the sample was injected, a vacuum diverter was set to 40 sec with an automatic timer in order to prevent the solvent peak from entering the ion source. A six-channel programmable multiple-ion monitor (PROMIM) was used for selected ion monitoring by focusing on the most prominent ions.

The following standards were obtained from commercial sources and were used without further purification: phenylethylene glycol (PEG), Pfaltz and Bauer, Stamford, Conn., U.S.A.; *p*-hydroxyphenylethanol (pHPE), *m*-hydroxyphenylethanol (mHPE), *o*-hydroxyphenylethanol (oHPE) and *p*-hydroxyphenylpropanol (pHPP), Aldrich, Milwaukee, Wisc., U.S.A.; 3-methoxy-4-hydroxyphenylethanol (MHPE) and 3,4-dihydroxyphenylethanol (DHPE), Regis, Morton Grove, Ill., U.S.A.; and 3,4-dihydroxyphenylethylene glycol (DHPG), Sigma, St. Louis, Mo., U.S.A. *p*-Hydroxyphenylethylene glycol (pHPG) was synthesized by Dr. B.L. Goodwin.

RESULTS

Table I shows the CI mass spectra obtained with methane as the reagent gas for the PFP derivatives of several representative neutral alcoholic metabolites. The derivatives of all the metabolites except DHPE had their base peaks corresponding to the loss of pentafluoropropionic acid (*m/e* 164) from the M+1 ions. These ions appeared at *m/e* 267 for PEG and pHPE, *m/e* 281 for pHPP, *m/e* 429 for pHPG, *m/e* 591 for DHPG, *m/e* 297 for MHPE and *m/e* 459 for MHPG. The base peak of the derivative of DHPE resulted from the loss of a second PFP group in addition to PFPOH. The striking feature of these CI mass spectra is the appearance in most cases of only a single prominent ion in the mass spectra. Thus, for PEG, pHPE, DHPE and MHPE, no peak exceeded 4% of the abundance of the base peak. The M+1 ions were detectable in the spectra of only three of the derivatives, and then as peaks with a relatively low abundance: pHPP (1%), MHPE (2%), and MHPG (9%).

In order to reduce the fragmentation of the M+1 ions, we obtained CI spectra using isobutane as the reagent gas. Although in this case the relative abundance of the M+1 ions of MHPE and MHPG increased to 14% and 35%, respectively, still no M+1 ion was detected for PEG. However, because of practical considerations [10], we chose to use methane as the reagent gas for all further studies.

Fig. 1 shows the selected ion-current profiles obtained for a mixture of eight derivatives separated on three different columns. The retention times observed are summarized in Table II. The order by which the compounds eluted was the same on each of the three columns, except that MHPG eluted before MHPE on 3% SP-2250 and that DHPG eluted after both MHPE and MHPG on 3% QF-1.

TABLE I

CI MASS SPECTRA OF THE PFP DERIVATIVES OF SOME PHENYLGLYCOLS AND PHENYLETHANOLS

Mass spectra were obtained at 90 eV by scanning over the range from *m/e* 180 to *m/e* 800. Minor peaks (usually < 1% abundance) and isotope peaks are not shown. Abbreviations are defined in the Experimental section. The mass spectra for mHPE and oHPE were virtually identical to that of the isomer, pHPE (shown below) with base peaks at *m/e* 267 and peaks at *m/e* 295 and 307 equal to 3% abundance. mHPE, but not oHPE, had a peak at *m/e* 247 of 3% abundance.

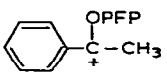
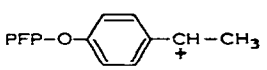
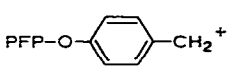
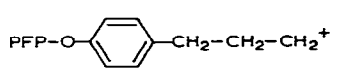
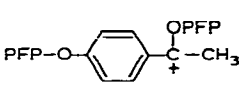
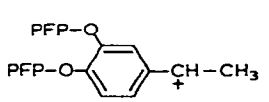
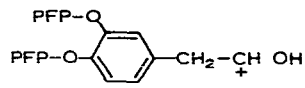
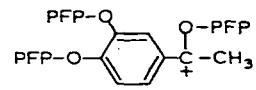
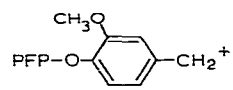
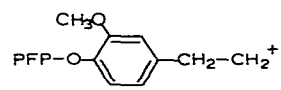
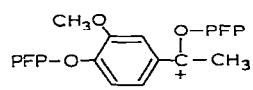
Compound	*m/e*	Relative abundance	Probable structure
PEG	267	100	
	295	3	(M — PFPOH) + $C_2H_5^+$
	307	2	(M — PFPOH) + $C_3H_5^+$
pHPE	247	4	*m/e* 267 — HF
	267	100	
	295	4	(M — PFPOH) + $C_2H_5^+$
	307	4	(M — PFPOH) + $C_3H_5^+$
pHPP	253	24	
	281	100	
	309	4	(M — PFPOH) + $C_2H_5^+$
	321	4	(M — PFPOH) + $C_3H_5^+$
	425	1	(M+1) — HF
	445	1	M+1
pHPG	267	15	*m/e* 429 — (OPFP) + 1
	429	100	
DHPE	263	2	*m/e* 283 — HF
	283	100	*m/e* 429 — (PFP) + 1
	311	4	*m/e* 429 — (PFP) + $C_2H_5^+$
	323	3	*m/e* 429 — (PFP) + $C_3H_5^+$
	429	2	

TABLE I (*continued*)

Compound	*m/e*	Relative abundance	Probable structure
	445	1	
DHPG	267	2	*m/e* 429 — (OPFP) + 1
	283	5	*m/e* 429 — (PFP) + 1
	429	10	*m/e* 591 — (OPFP) + 1
	445	2	*m/e* 591 — (PFP) + 1
	542	8	—
	591	100	
MHPE	277	3	*m/e* 297 — HF
	283	2	
	297	100	
	325	4	(*m/e* 297 — 1) + $C_2H_5^+$
	337	1	(*m/e* 297 — 1) + $C_3H_5^+$
	441	1	(M+1) — HF
	461	2	M+1
MHPG	297	14	*m/e* 459 — (OPFP) + 1
	411	9	—
	459	100	
	623	9	M+1

Urine specimens were analyzed on both 3% QF-1 and 3% OV-1. Equivalent results were obtained on either column. Since pHPG also produced a fragment at *m/e* 267 with an abundance 15% of the base peak (Table I), the appearance of this peak confirmed the identity of pHPG in these samples.

The results of analyses of five separate 24-h urine collections from the same patient are given in Table III. The average excretion of free + conjugated PEG, pHPE, pHPG and MHPG during the drug-free period was 22, 11, 91 and 1399 μg/24 h, respectively. DHPG and MHPE were also detected in these samples, but they were not quantitated. Treatment with a monoamine oxidase inhibitor resulted in a diminished excretion of pHPG and MHPG (to 30% and 14%, respectively, of the drug-free excretion level) but not of PEG and pHPE. The selected ion-current profiles for two of these samples, one obtained during the drug-free period and one during treatment with the monoamine oxidase inhibitor, are shown in Fig. 2.

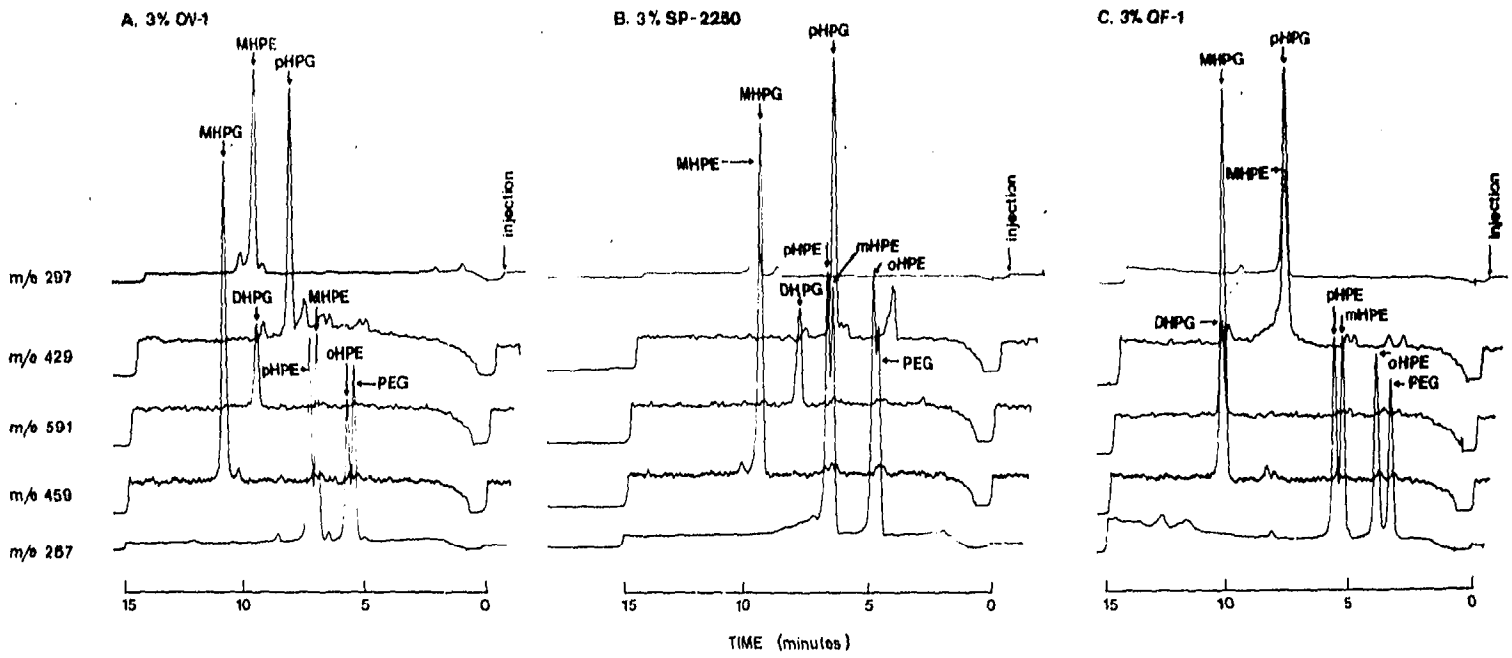


Fig. 1. Selected ion profiles for a standard mixture separated on: A, 3% OV-1 (programmed from 90° at 6°/min); B, 3% SP-2250 (from 90° at 6°/min); and C, 3% QF-1 (from 120° at 6°/min). The recorder was set at 2 V for the top channel (except for panel A, 1 V), 200 mV for each of the second, third and fourth channels, and 2 V for the bottom channel.

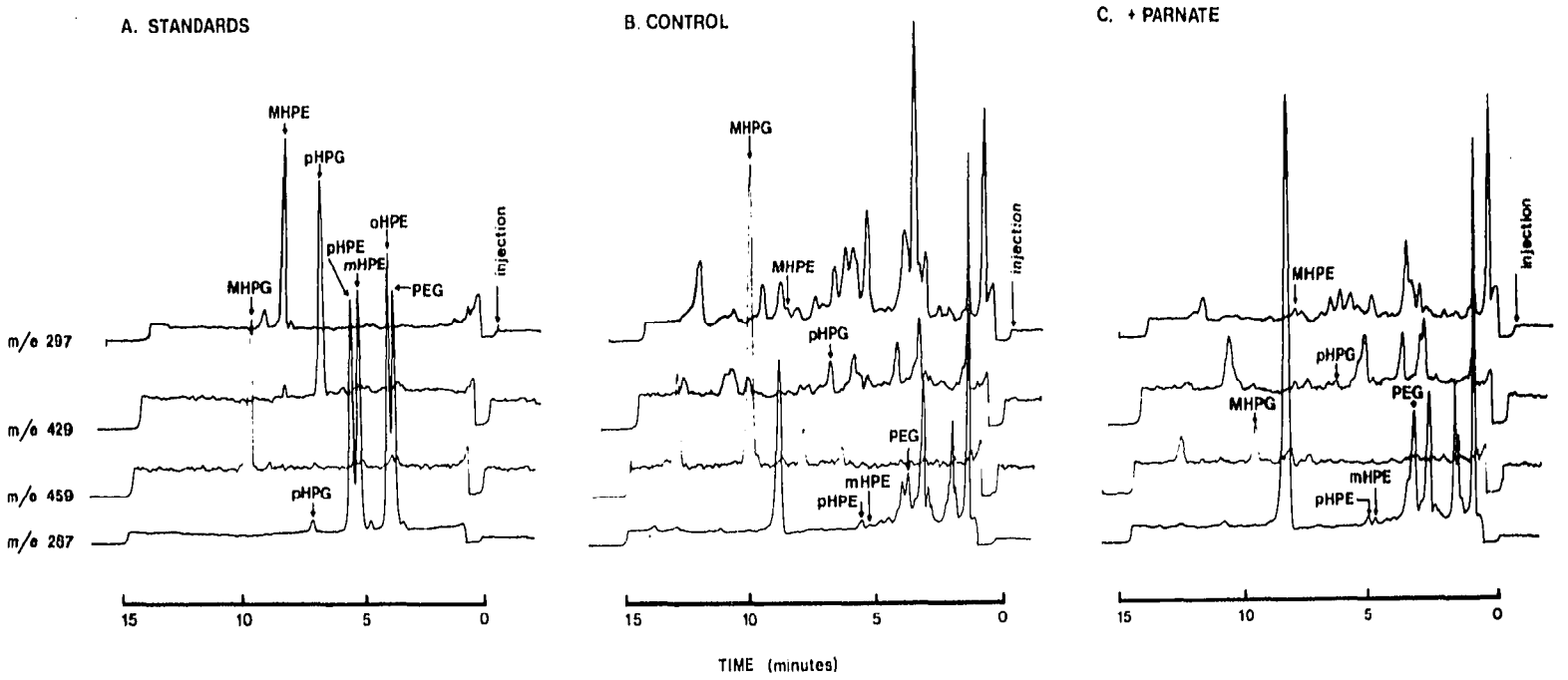


Fig. 2. Selected ion profiles for the analysis of phenylglycols and phenylethanols in urine specimens obtained from a patient drug-free and during treatment with a monoamine oxidase inhibitor (40 mg/day Parnate). A, Mixture containing 0.44 ng/μl of each standard (unextracted). Injection volume = 2 μl. B, Urine specimen obtained on day 1, drug-free (see Table III). C, Urine specimen obtained on day 48, during Parnate treatment. A 3% OV-1 column was used and programmed from 90° at a rate of 4°/min. The recorder was set for the channels from top to bottom at 500 mV, 200 mV, 200 mV and 1 V.

In separate aliquots of these urine specimens, the concentrations of the free metabolites were determined without treatment with glusulase. The average excretion of free pHPG and MHPG was 13 and 19 μg/24 h, respectively, or 14% and 1% of the total excretion of these metabolites. No free pHPE could be

TABLE II

RETENTION TIMES OF THE PFP DERIVATIVES OF PHENYLGLYCOLS AND PHENYLETHANOLS ON 3% OV-1, 3% SP-2250 AND 3% QF-1

All retention times are given in minutes.

Compound	Column		
	3% OV-1 (90°+ 6°/min)	3% SP-2250 90° + 6°/min)	3% QF-1 (120° + 6°/min)
PEG	5.41	4.56	3.30
oHPE	5.70	4.78	3.88
mHPE	6.96	6.38	5.33
pHPE	7.26	6.58	5.67
pHPG	8.60	6.93	8.28
DHPG	9.73	8.01	10.55
MHPE	10.39	10.32	8.62
MHPG	10.94	9.48	10.32

TABLE III

URINARY EXCRETION OF TOTAL NEUTRAL METABOLITES IN A SUBJECT BEFORE AND DURING TREATMENT WITH PARNATE (40 mg/day)

Parnate (®, Smith, Kline and French; tranylcypromine) was administered beginning on day 33.

Day	Parnate dose (mg/day)		PEG	pHPE	pHPG	MHPG
μg/24 h						
1	—		15	14	108	1680
4	—		28	13	145	1396
5	—		22	6	21	1121
		Mean	22	11	91	1399
48	40		32	10	18	144
49	40		22	17	35	254
		Mean	27	14	27	199
			(125%)	(127%)	(30%)	(14%)
μg/g creatinine						
1	—		8	7.4	57	893
4	—		15	7.0	78	755
5	—		12	3.3	12	623
		Mean	12	5.9	49	757
48	40		30	9.4	17	135
49	40		10	7.9	16	117
		Mean	20	8.7	17	126
			(171%)	(147%)	(34%)	(17%)

detected in these urine specimens, nor could either free pHPG or MHPG be detected in urines obtained during treatment with the monoamine oxidase inhibitor.

DISCUSSION

The alcoholic metabolites formed from biogenic amines are present in urine in significant amounts. Measurement of the concentrations of these metabolites in urine or tissues may be necessary in order to gain a complete picture of how these amines are metabolized and what are the effects of drugs. This may be particularly true for the β-hydroxylated phenylethylamines, since these compounds appear, at least in brain tissue, to be converted predominately to the corresponding neutral metabolite (i.e., the phenylglycol) rather than to an acidic one [11].

The present report provides a highly specific and sensitive method for the determination of alcoholic metabolites of phenylethylamines. The only limitation to the number of metabolites which can be analyzed simultaneously is the number of PROMIM channels available. The use of at least two different columns or in some cases monitoring two ion fragments insures that the peaks are correctly identified.

Quantitation was achieved by measuring peak heights and comparing them to standards carried through the extraction scheme. Plotting the peak heights against the amount of standard added resulted in linear relationships. In some cases, we added pHPP as an internal standard. Results obtained by measuring peak height ratios using the internal standard were virtually identical to those obtained by simply measuring the peak heights. The calibration curves using the internal standard were not improved, and in fact were slightly worse than those obtained without the internal standard. For this reason and because the internal standard required an additional PROMIM channel, we have routinely carried out our analyses without an internal standard.

The identification of pHPG in human urine to our knowledge represents the first demonstration of this metabolite in biological samples. The excretion of this metabolite, which is the β-hydroxylated analogue of pHPE, not surprisingly was 8-fold greater than pHPE. Nevertheless, compared to MHPG, the excretion of pHPG was only 7% as much. On the other hand, the concentration of octopamine, the amine precursor of pHPG, is exceedingly low in most tissues [12]. The concentration in brain, for example, is about 5 ng/g [12], or only 1% of the precursor of MHPG, norepinephrine. Taken together, these results support the suggestion based on the 5- to 10-fold increase in octopamine concentrations following treatment with a monoamine oxidase inhibitor that although the concentration of octopamine in tissues is very low, the turnover rate of this amine is nevertheless relatively large [12]. Our results suggest that the determination of pHPG excretion may provide a means of measuring the turnover of octopamine in vivo.

Treatment with 40 mg per day of the monoamine oxidase inhibitor, Parnate, resulted in a 70% reduction in the excretion of pHPG. In comparison, the excretion of MHPG was reduced by 86%, but no reduction was observed in the excretion of either PEG or pHPE. The cause of each of the deaminated neutral

metabolites not being reduced to the same extent is not known. One possible explanation is based on the fact that there are two forms of monoamine oxidase which differ in their substrate specificities and inhibitor sensitivities [13]. Norepinephrine is a specific substrate for type A monoamine oxidase [13], and octopamine is deaminated primarily by type A monoamine oxidase but is deaminated to some extent by type B monoamine oxidase [14]. Tyramine is deaminated by both forms of the enzyme, whereas phenylethanolamine is a specific substrate for type B monoamine oxidase [8]. Thus, the more specific a substrate is for the A form of monoamine oxidase, the greater is the reduction produced by Parnate in the excretion of the corresponding deaminated metabolite. The effect of Parnate on these metabolites would be explained if this drug were selectively blocking type A monoamine oxidase in vivo. However, there is no direct evidence for this, and in fact in vitro Parnate is nonspecific, inhibiting each form of the enzyme with about an equal potency [13]. These results could be explained alternatively if the drug produces a different degree of inhibition at different cellular locations. Thus, since norepinephrine and octopamine are presumed to be deaminated mainly within noradrenergic neurons, an accumulation of the drug by these neurons could account for the larger decrease in MHPG and pHPG formation.

Since this report was written, we have identified and quantitated pHPG in both rat brain and urine. Sprague—Dawley rats (300—350 g) were found to excrete (mean ± S.E.) 0.12±0.03 and 4.8±0.9 μg/24 h of free and total pHPG, respectively. The whole brain concentration of total pHPG was 3.1±0.6 ng/g [15].

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