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THE 4-*O*-METHYL METABOLITES OF CATECHOLAMINES. HOMO-*ISO*-VANILLIC ACID IN RAT URINE AND BRAIN; URINARY *ISO*-VANYL COMPOUNDS AFTER INTRAPERITONEAL ADMINISTRATION OF DOPAMINE AND OF DOPAMINE PRECURSORS AND DERIVATIVES*

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Abstract—Homo-*iso*-vanillic acid (3-hydroxy, 4-methoxyphenylacetic acid, *iso*-HVA) was detected in rat urine and brain, with a molar ratio of *iso*-HVA to HVA of 0·07 in urine and about 0·35 in brain. The free urinary *iso*-vanyl and vanyl phenolcarboxylic acids were studied after intraperitoneal loads with the following compounds: L-dopa, L-3-*O*-methyldopa, L-4-*O*-methyldopa, dopamine, 3-*O*-methyldopamine, 4-*O*-methyldopamine and 3,4-dihydroxyphenylacetic acid. The results suggest the following conclusions. (1) The molar ratio *iso*-HVA/HVA is not constant. After dopa as well as dopamine loads it rises with the increase of the dose of precursor administered, showing that, *in vivo*, the 4-*O*-methylation process depends, to some extent, on the substrate concentration. (2) In contrast with the other catechols (dopamine and 3,4-dihydroxyphenylacetic acid), L-dopa itself does not seem to be *para*-*O*-methylated. It is therefore unlikely that 4-*O*-methyldopa would be a metabolite of L-dopa *in vivo*. (3) Distinct urinary metabolites from L-3-*O*-methyldopa (vanylmandelic and vanillic acids) on one hand and from L-4-*O*-methyldopa (unknown *iso*-vanyl phenolcarboxylic acid) on the other, support evidence that some of the metabolic transformations of the side-chain of the *O*-methyl-catecholamines are different according to whether the methyl group is bound on the *meta* or on the *para* position. (4) The high level of cerebral *iso*-HVA might be due to either a lower *iso*-HVA than HVA transport outside the brain, or to the existence, in addition to the dopamine source, of a second cerebral metabolic pathway for the production of *iso*-HVA.

The *O*-methylation reactions of catecholamines are implicated in some neuropsychiatric disorders. Thus, it has been suspected that *para*-*O*-methylation of dopamine occurs in schizophrenia, as a possible source of psychotoxic derivatives such as 3,4-dimethoxyphenylethylamine [1, 2]. 4-*O*-Methyldopamine was recently suggested to be the endogenous "toxin" in Parkinsonism [3].

The *para*-*O*-methylation pathway was regarded as a disordered metabolism until the identification of 4-*O*-methyl derivatives of the catecholamines in healthy human subjects by Mathieu and Revol [4, 5]. Among them, homo-*iso*-vanillic acid (3-hydroxy,4-methoxyphenylacetic acid, *iso*-HVA) has been detected in urine [5–7], in human CSF [8, 9] as well as in animal brain [10]. In human urine, the molar ratio of *iso*-HVA to HVA was low (about 0·07), but it was found to be greater (about 0·5) in the brain (caudate nucleus) of the sheep [10].

In the present paper we report the identification of homo-*iso*-vanillic acid in the urine and brain of the rat, and the determination of the respective *iso*-HVA/HVA ratios. After *in vivo* loads with precursors, metabolites of dopamine and related 3- and 4-*O*-methyl derivatives, we also studied the urinary vanyl and *iso*-vanyl phenolcarboxylic acids. This work was initiated to obtain information about the *in vivo* formation and metabolism of the 4-*O*-methyl derivatives of catecholamines.

MATERIALS AND METHODS

Reagents. The following compounds were obtained from commercial sources: L-dopa, dopamine, 3,4-dihydroxyphenylacetic acid and homovanillic acid (Fluka); 3- and 4-*O*-methyldopamine (Regis Chem.) and most of the phenolic acids used as standards for paper

* List of abbreviations: 3-Mdopa, 4-Mdopa = L-3- and L-4-*O*-methyldopa; DA = dopamine; 3-MDA, 4-MDA = 3- and 4-*O*-methyldopamine; HVA = 4-hydroxy,3-methoxyphenylacetic acid, homovanillic acid; *iso*-HVA = 3-hydroxy,4-methoxyphenylacetic acid, homo-*iso*-vanillic acid; DOPAC = 3,4-dihydroxyphenylacetic acid; VLA, *iso*-VLA = vanyllactic and *iso*-vanyllactic acid; COMT = catechol-*O*-methyltransferase (EC 2.1.1.6); DzNA = diazotized paranitroaniline.

chromatography. L-3- and L-4-*O*-methyldopa were the generous gifts of Hoffmann–Laroche Lab., Basle (Switzerland). Homo-*iso*-vanillic acid and *iso*-vanylpropionic acid were synthesized in our laboratory according to a previously described procedure [5]. *m*-Hydroxyphenylpropionic acid was prepared by hydrogenation of *m*-hydroxycinnamic acid (Fluka) with zinc amalgam and concentrated hydrochloric acid, according to Kemp [11].

Animals. Albino rats of both sexes, Sprague–Dawley strain, 200–300 g body wt, were used. Forty-eight hr before, and during the experiments, they had free access to water and sucrose only. All compounds tested were administered (from 1 to 20 mg/100 g body wt) by intraperitoneal injection after being dissolved in distilled water (DA, 3-MDA, 4-MDA, DOPAC) or in 0·05 N HCl (dopa, 3-M dopa, 4-M dopa). The 24 hr post-injection urines were collected in 2 ml 1·0 N hydrochloric acid in glass metabolism cages, and the free phenolic acids were immediately extracted.

Animals were killed by decapitation and the brains were rapidly removed and kept frozen at −20° until homogenized.

Paper chromatography of the urinary phenolic acids. After checking the pH (indicator paper) which must be 1–2, the whole 24 hr urine samples (usually a volume of 40–65 ml) were saturated with NaCl. The free phenolic acids were extracted 3 times with 30 ml ethyl acetate, then transferred in 10% aqueous $KHCO_3$ (3 extractions with 10 ml each). The alkaline solution, brought to pH 1 and saturated with NaCl, was extracted 3 times with 10 ml ethyl acetate. The extract was dried over anhydrous sodium sulphate for 24 hr. For controls, the whole of the organic extract was evaporated to dryness and transferred to Whatman No. 20 paper for a bi-dimensional chromatographic separation. After i.p. loads, 1/10th only of the extract was chromatographed on one paper sheet. The following solvents were used: 1st direction, descending development in isopropanol–ammonia–water (8:1:1, by vol.); 2nd direction, ascending development in benzene–propionic acid–water (100:70:4, by vol.). The sheets were air-dried for 24 hr to remove the acid completely. Ultraviolet light (254 nm) was always used to detect fluorescent spots on chromatograms before spraying. Diazotized *p*-nitroaniline was made according to Mathieu and Revol [12] and lightly sprayed on both faces of the chromatograms. Gibbs' reagent (2,6-dichloroquinone-4-chloroimide) was useful for the observation of *iso*-vanyl compounds [7, 13]. *p*-Dimethylaminobenzaldehyde in acetic anhydride (4% w/v) was used for hippuric acids (glycine conjugates) [14].

In some cases, a comparison was made between chromatograms before and after hydrolysis of the urine (in 20% HCl, for 2 hr under a reflux) according to Booth *et al.* [15] for the appearance of new areas, or the disappearance of other areas.

Paper chromatography of the phenolic acids from the brain tissue. The pooled brains (2 or more) were homogenized in 5 parts (w/v) of ice-cooled 0·4 N perchloric acid in a Potter homogenizer. The homogenate, plus 5 ml 0·4 N $HClO_4$ used for washing the apparatus, was centrifuged at 0° at 20,000 rev/min for 15 min. The supernatant was collected and the pellet washed with 5 ml 0·4 N $HClO_4$ and centrifuged again. The pooled supernatants were brought to pH 6 with 20% (w/v) KOH, added drop by drop. The perchlorate precipitate was discarded by centrifugation and the clear fluid was brought to pH 1 with 6N HCl, then saturated with NaCl. The phenolic acids were extracted three times with ethyl acetate, then transfered in 10% w/v aqueous $KHCO_3$ (3 extractions with 3 ml each). The alkaline solution, brought to pH 1 and saturated with NaCl, was extracted with ethyl acetate (5 ml, 3 times). The organic phase was dried over anhydrous sodium sulphate for 24 hr, then evaporated to dryness and transferred without loss to Whatman No. 20 paper. Standards (HVA and *iso*-HVA, 1 μg each) were spotted on the same paper sheet and run beside the extracts in both directions. 1st solvent; isopropanol–ammonia–water (8:1:1, by vol.), descending development: 2nd solvent; isopropanol–acetic acid–water (8:1:1, by vol.), ascending development. After 24 hr air drying, both faces of the paper were lightly sprayed with diazotized paranitroaniline.

Chromatographic separation of the 3- *and* 4-*O-methyl isomers.* The vanyl and *iso*-vanyl compounds usually exhibit identical chromatographic mobilities (R_f values), but can be separated as their azo-derivatives with diazotized paranitroaniline (DzNA). The procedure for this separation involves cutting out the spot area, elution in a small volume of methanol (30–50 μl) in a closed tank, and chromatography of the azo-dyes. The method and details of this procedure have been described previously [9, 10]. The following solvents, ascending, were used for the bi-dimensional separation of the azo-derivatives: 0·1 M Na_2CO_3–ethyl alcohol–ammonia (8:1:1, by vol.) (1st development) and isopropanol–0·1 M Na_2CO_3–ammonia (4:5:1, by vol.) (2nd development). By this procedure, the azo-derivatives of HVA (grey-blue) and *iso*-HVA (violet) can be completely separated from each other. For R_f values of the azo-derivatives of the vanyl and *iso*-vanyl compounds, see Mathieu *et al.* [9].

Quantitative evaluation. In order to get a quantitative estimation of HVA and *iso*-HVA, 0·05–20 μg of these compounds were chromatographed and treated in the same way as the corresponding spots from biological extracts. Each spot from the urinary extract was evaluated by visual comparison with three different ranges of standards. When different values were obtained the mean was retained. In the case of large excretion of one or other metabolite, only measured aliquots of the methanolic eluates were chromatographed, with the object of obtaining spots coming within the standard scales. Recovery studies were made with both HVA and *iso*-HVA. The recovered levels were found to range from 65 to 82 per cent (mean 71%) for HVA, and from 62 to 80 per cent (mean 69%) for *iso*-HVA.

RESULTS

*Homovanillic and homo-*iso*-vanillic acids in the urine and brain of the fasting rat.* Figure 1 is a schematic representation of the chromatogram of the urinary phenolic acids from the fasting rat. Ten specimens of urine were analysed, the results being quite similar. The listed phenolic acids agree with previous identifications by other workers, especially by Borud *et al.* [16]. Free vanylmandelic acid was not detected, although Caesar *et al.* [17] have reported the presence of small amounts of this compound in the urine of the rat. Free homovanillic acid was always present.

The daily HVA excretion was found to be 24–54 μg/kg, mean value (9 assays) = 48 μg/kg. Homo-*iso*-vanillic acid was detected in each of the urinary samples analyzed, with daily excretion ranging from 1 to 4·5 μg/kg, mean value (9 assays) = 3·4 μg/kg. *Iso*-HVA represented 6·5 per cent of the total value of *iso*-HVA + HVA. The molar ratio *iso*-HVA/HVA was thus 0·07.

Due to the low levels of compounds present in the brain of control rats, no visible spots were detected on the chromatograms either from one brain or from two brains pooled. From ten brains pooled (2 assays), several distinct spots were observed, one of which had the same chromatographic characters (colour and R_f values) as the HVA + *iso*-HVA standard. After elution and rechromatography, HVA and *iso*-HVA were separated into distinct spots and identified. The 4-*O*-methyl isomer was estimated to be present in about 25 per cent of the total value of *iso*-HVA + HVA. The molar ratio of *iso*-HVA/HVA was near 0·35.

The quantitative estimations of the urinary HVA and *iso*-HVA excretions during the 24 hr after the different i.p. loads are presented in Table 1.

Urinary metabolites after the loads with 3,4-*dihydroxyphenyl derivatives*: L-*dopa, dopamine and* 3,4-*dihydroxyphenylacetic acid.* L-Dopa was administered at the doses of 1 mg, 2·5 mg, 5 mg, 10 mg and 20 mg/100 g body wt. In each of these assays, the most elevated metabolites were homovanillic acid and homo-*iso*-vanillic acid. Vanyllactic acid was also present, but to a smaller extent, similar to what was described by O'Gorman *et al.* [7] in man given L-dopa.

It may be seen from Table 1 that with the increase of the dose of dopa administered, both the individual levels of HVA and *iso*-HVA and the percentage of *iso*-HVA increase: it rises from 6·5 per cent in controls without dopa, to 50 per cent after dopa, 20 mg/100 g body wt.

Neither the elution and rechromatography of the corresponding coloured spot, nor spraying with Gibbs' reagent showed the presence of *iso*-vanyllactic acid in addition to vanyllactic acid.

After dopamine administration (1 mg and 5 mg/100 g body wt) the urinary levels of HVA and *iso*-HVA were found to be higher than after the corresponding L-dopa doses but, unexpectedly, these metabolites were reduced after the 10 mg dopamine load. As for dopa, the rise of the *para/meta* isomers ratio followed that of the dose of dopamine injected.

After DOPAC (20 mg/100 g body wt), very strong spots of homovanillic and homo-*iso*-vanillic acids were observed, but the percentage of *iso*-HVA was found to be smaller (32%) than after a corresponding dose of L-dopa (50%) and after a smaller (one-half) dose of dopamine (55%).

Urinary metabolites after the loads with 3-*methoxy*, 4-*hydroxy* (*vanyl*) *derivatives*: 3-O-*methyldopa and* 3-O-

Table 1. Urinary excretion of homovanillic and homo-*iso*-vanillic acids during the 24 hr after intraperitoneal administration to the rat of precursors, metabolites of dopamine, and related 3- and 4-*O*-methyl derivatives

Substance	Dosage (mg/100 g body wt)	Homovanillic acid		Homo-*iso*-vanillic acid	
		μg/24 hr†	%‡	μg/24 hr†	%‡
Controls (9)*		11·5 (48 μg/kg)	93·5	0·8 (3·4 μg/kg)	6·5
L-Dopa (2)	1	50	83·5	10	16·5
L-Dopa (2)	2·5	75	62·5	45	37·5
L-Dopa (2)	5	75	60	50	40
L-Dopa (4)	10	100	54	85	46
L-Dopa (9)	20	200	50	200	50.
L-3-*O*-Methyldopa (2)	10	90	(100)	(+)	
L-3-*O*-Methyldopa (4)	20	200	96	9	4
L-4-*O*-Methyldopa (2)	10	(+)		>400	(100)
Dopamine (2)	1	90	78	25	22
Dopamine (2)	5	130	50	130	50
Dopamine (2)	10	75	45	90	55
3-*O*-Methyldopamine (2)	10	>400	(100)	ND	
4-*O*-Methyldopamine (2)	10	ND		400	(100)
3,4-Dihydroxyphenylacetic acid (2)	20	400	68	125	32.

* The number of experiments (one animal in each experiment) is indicated in parentheses. † Each value is the mean of the determinations.

‡ Per cent of the total *iso*-HVA + HVA value. ND = not detected. (+) = traces. (100) = close to 100%.

methyldopamine. After a load with 3-*O*-methyldopa 10 mg/100 g body wt, spots of the substance administered (3-Mdopa) and of two strongly enhanced spots could be observed: those of homovanillic and vanyllactic acids. Vanillic acid also was elevated, but to a small extent. Trace amounts of homo-*iso*-vanillic acid were found, but none of *iso*-vanyllactic acid. After a 20 mg/100 g body wt load, a larger amount of *iso*-HVA was detected.

Administration of 3-*O*-methyldopa also resulted in the appearance of a spot having the same characters (R_f values and colour with DzNA) as vanylmandelic acid. This spot, although much smaller than those of HVA and VLA, was eluted and co-chromatographed with the azo-derivative of authentic VMA. The two compounds were found to have identical mobilites (R_f values) in the solvents described above. Gjessing [18, 19] suggested that such vanylmandelic acid was formed due to a decomposition of vanylpyruvic acid in the alkaline chromatographic run. This was tested by further assays (3-M dopa, 20 mg/100 g body wt, two assays) during which the urinary extracts were prepared without alkaline washing, and the phenolic acids were separated by monodimensional acidic run, according to a procedure described by Mathieu and Revol [12]. These assays confirmed the presence of free VMA in rat urine after 3-Mdopa administration, which could therefore be assumed to have an *in vivo* origin (*in vivo* decarboxylation of vanylpyruvic acid?). This finding led us to reexamine our chromatograms after the L-dopa loads, where we detected traces of vanylmandelic acid.

After 3-*O*-methyldopamine (10 mg and 20 mg/100 g body wt) we could observe only one strongly elevated spot of HVA, without *iso*-HVA.

Urinary metabolites after loads with 3-*hydroxy*, 4-*methoxy* (iso-*vanyl*) *derivatives*: 4-O-*methyldopa and* 4-O-*methyldopamine*. The most prominently elevated metabolite after the 4-*O*-methyldopa loads (10 mg/100 g body wt, 2 assays) was homo-*iso*-vanillic acid. Two other large spots were seen, corresponding to *iso*-vanyllactic acid and to an unknown compound (spot no. 19, Fig. 1). This compound was destroyed by acid hydrolysis; a coloured reaction with Gibbs' reagent indicated an *iso*-vanyl structure but sprays with *p*-dimethylaminobenzaldehyde for glycine conjugates and 2,4-dinitrophenylhydrazine [20] for the pyruvic acids were negative. Owing to its position and colour this compound was compared with 3-hydroxy, 4-methoxyphenylhydracrylic acid by elution and co-chromatography with the azo-derivative of the authentic substance. The two azo-derivatives exhibited different chromatographic characters. Neither was spot no. 19 found to be identical to *iso*-vanylpropionic acid. Two further assays were made in the same way as for 3-Mdopa (see above) to avoid any pyruvic acid decomposition to form spot no. 19, as an *in vitro* artifact. They resulted again in the observation of this spot. This compound (no. 19), which appears as a strong urinary *iso*-vanyl metabolite after i.p. administration of 4-Mdopa to the rat, has therefore not been identified. In addition, minute amounts of *iso*-vanylmandelic acid were observed. Careful examination of our chromatograms after the 3-Mdopa loads showed trace amounts of the vanyl counterpart of spot no. 19.

After 4-*O*-methyldopamine (10 mg and 20 mg/100 g body wt), *iso*-HVA was the only strongly elevated spot.

Lastly, it appeared that despite the fact that the *iso*-vanyl isomers react more intensively with diazotized

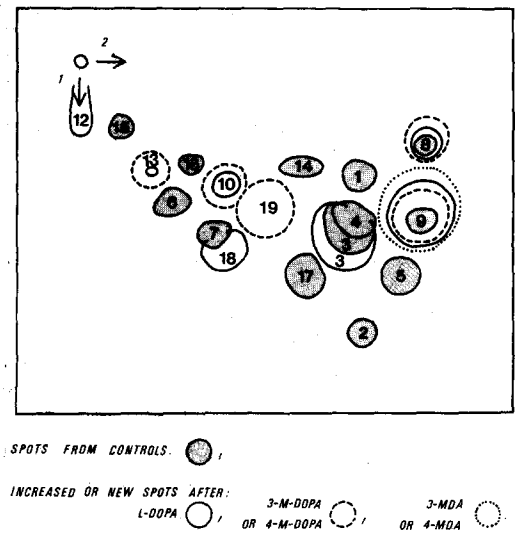

Fig. 1. Bidimensional paper chromatogram of the free phenolic acids from rat urine after a 48 hr fasting (dotted spots), and after i.p. administration of several precursors and derivatives of dopamine.

Whatman No. 20 paper; solvent 1 = isopropanol–ammonia–water (8:1:1, by vol.), descending development; solvent 2 = benzene–propionic acid–water (100:70:4, by vol.), ascending development; detection of the spots under u.v. light (254 nm) and with diazotized *p*-nitroaniline (DzNA).

1 = *p*-hydroxybenzoic acid (u.v. = dark blue, DzNA = pink); 2 = *O*-hydroxyphenylacetic acid (pink–purple); 3 = *m*-hydroxyphenylacetic acid (pink); 4 = *p*-hydroxyphenylacetic acid (lilac); 5 = *m*-hydroxyphenylpropionic acid (u.v. = dark blue, DzNA = pink); 6 = *p*-hydroxymandelic acid (pink); 7 = *p*-hydroxyphenyllactic acid (lilac); 8 = vanillic acid (violet); 9 = homovanillic acid (grey–blue) and homo-*iso*-vanillic acid (violet); 10 = vanyllactic acid (grey–blue) and *iso*-vanyllactic acid (violet); 11 = 3,4-dihydroxyphenylacetic acid (pink); 12 = L-dopa (u.v. = orange, DzNA = light pink, partly destroyed in alkaline run); 13 = vanylmandelic acid (violet) after 3-Mdopa, traces after dopa loads, and *iso*-vanylmandelic acid (violet), traces after 4-Mdopa load; 14 = *O*-hydroxyhippuric acid (u.v. = light blue, DzNA = pink); 15 = xanthurenic acid (u.v. = light blue, DzNA = purple); 16 = 5-hydroxyindole, 3-acetic acid (u.v. = blue, DzNA = pink); 17 = hippuric acid (u.v. = dark blue, DzNA = 0); 18 = *m*-hydroxyhippuric acid (u.v. = blue, DzNA = red); 19 = unknown *iso*-vanyl compound after 4-Mdopa load (DzNA = violet, split by acidic hydrolysis), traces of the vanyl counterpart (DzNA = blue) after 3-Mdopa load.

p-nitroaniline, the spots of the urinary metabolites, after the loads with *iso*-vanyl compounds, were larger (about twice as large or even more: see Table 1, after 3- and 4-Mdopa, 10 mg/100 g body wt) than those of the corresponding metabolites after administration of the vanyl compounds.

DISCUSSION

Homo-*iso*-vanillic acid is present in the urine of the rat, with a daily excretion between 1 to 4·5 μg/kg, mean of 9 assays = 3·4 μg/kg. Using the same chromatographic procedure, the daily excretion of HVA was between 24 and 54 μg/kg, mean of 9 assays = 48 μg/kg (31·97 ± 4·07 μg/kg was indicated by Caesar *et al.* [17]). The molar *iso*-HVA/HVA ratio in the rat urine is thus 0·07, which is the same value as that found in human urine by Mathieu *et al.* [10].

Homo-*iso*-vanillic acid was also detected in the rat brain with a molar *iso*-HVA/HVA ratio near 0·35, close to that of about 0·5 found in the brain (caudate nucleus) from sheep [10].

Several factors emerge from the examination of the chromatograms of the urinary phenolic acids following the different intraperitoneal loads.

First, the molar ratio *iso*-HVA/HVA did not appear to be a constant. After dopa as well as dopamine loads, it rose with the increase of the dose of precursor administered, showing that, *in vivo*, the 4-*O*-methylation process depends to some extent, on the substrate concentration. This is in accordance with previous observations of a higher molar ratio *iso*-HVA/HVA than the normal one in the urine from patients with L-dopa therapy or with neuroblastoma [5, 8 and personal unpublished data]. That an increase of the substrate enhances the 4-*O*-methylation pathway, which is regarded as a possible source of psychotoxic derivatives [21], might have some physiological and/or pathological incidence, for instance under stress conditions during which an increase of the free catecholamines may locally occur.

After the L-dopa loads we noticed that while an *iso*-HVA increase always occurred, no *iso*-vanyllactic acid was ever detected. This seems to indicate that, *in vivo*, both L-dopa and its catabolic derivatives which lead to VLA (i.e. 3,4-dihydroxyphenylpyruvic and 3,4-dihydroxyphenyllactic acids) do not undergo *para*-*O*-methylation, or in minute amounts, if any. It is therefore unlikely that 4-Mdopa would be an *in vivo* metabolite of L-dopa, while dopamine and DOPAC are partly *para*-*O*-methylated.

An increase in *iso*-HVA after 3-M dopa administration agrees with the previous suggestion that the 3-*O*-methylcatecholamine derivatives can be 3-*O*-demethylated *in vivo* to some extent, thus leading to the corresponding catecholamines. This was observed in the rat for metanephrine [22] and in the rat as well as in man, for 3-*O*-methyldopa [23–25]. The lack of any 4-*O*-methyl derivative increase after 3-*O*-methyldopamine, in opposition to 3-M dopa, is probably due to a very rapid deamination of the amine before any *O*-demethylation process starts.

Table 2 summarizes the main chromatographic modifications following the 3-Mdopa and 4-Mdopa loads. We note the presence of vanillic and vanylmandelic acids after 3-Mdopa, but the lack of their 4-*O*-methyl counterparts, or only traces, after 4-Mdopa administration. VMA was unexpected, and was not reported as a metabolite of 3-Mdopa in rat urine by Bartholini and Pletscher [24]. It might originate from *in vivo* decarboxylation of the corresponding pyruvic acid. However, the most prominent feature is the presence of a strong unidentified *iso*-vanyl compound (spot no. 19) after the 4-Mdopa loads, without its vanyl counterpart after 3-Mdopa. These findings suggest that not only the nature of the side-chain of the catechol derivatives influences the ratio in which they are *meta* and *para*-*O*-methylated by COMT, as reported by Senoh *et al.* [26], O'Gorman *et al.* [7] and Creveling *et al.* [27], but that afterwards some of the metabolic transformations of the side-chain of the *O*-methyl catecholamines are different according to whether the methyl group was bound on the *meta* or the *para* position. An urinary excretion rate of the end-metabolites apparently greater after the *iso*-vanyl than after the vanyl compounds loads could mean that a smaller part

Table 2. Urinary vanyl and *iso*-vanyl compounds after intraperitoneal administration of L-3-*O*-methyldopa and L-4-*O*-methyldopa

Compounds present on the chromatograms	After loading with L-3-*O*-Methyldopa	After loading with L-4-*O*-Methyldopa
Homovanillic acid	+++	+
Homo-*iso*-vanillic acid	(+)	++++
Vanyllactic acid	++	ND
iso-Vanyllactic acid	ND	++
Vanylmandelic acid	+	ND
iso-Vanylmandelic acid	ND	(+)
Vanillic acid	+	ND
iso-Vanillic acid	ND	ND
Unknown *iso*-vanyl compound (spot no. 19)	ND	++
Vanyl counterpart of spot no. 19	(+)	ND

ND = not detected; (+) = traces and + = present to ++++ = larger amounts.

of the 4-*O*-methyl escape observation than the 3-*O*-methyl metabolites due, in possible agreement with the former suggestion, to distinct metabolic routes. However, a greater renal clearance for *iso*-HVA than for HVA cannot be excluded.

It is of interest to compare some of our *in vivo* results with data from Creveling's comprehensive *in vitro* study of the *meta* and *para* methylation of catechol derivatives with purified COMT [27]. We suggested that, due to the lack of any *iso*-vanyllactic acid in the urine of the rat after L-dopa loads, L-dopa itself in opposition to its derivatives as dopamine and DOPAC was little or not 4-*O*-methylated. This agrees with these authors' *in vitro* finding that, while D-dopa gives a *meta*:*para* ratio of 3·4, the L isomer gives almost exclusively the *meta* methylation product. The *in vitro* value for dopamine (*meta*:*para* ratio at pH 7·0 = 10·2, at pH 8·1 = 6·9) is near to our *in vivo iso*-HVA/HVA ratio in the urine from control rats.

However our observation of a high *iso*-HVA/HVA ratio in the rat brain (0·35) contrasts with the finding, according to Creveling *et al.* [27] that *in vivo* the *para*/*meta* ratios would be lower than those obtained *in vitro*. Several hypotheses could therefore be considered to account for this high *iso*-HVA cerebral level, among which: (1) it could be a consequence of a lower transport of *iso*-HVA out of the brain than HVA, (2) in brain, *iso*-HVA would originate in both the dopa → dopamine pathway and in a second metabolic pathway. An hypothesis for such a second *iso*-HVA source could be through the formation of *O*-methyltyramine from tyramine (tyramine → *O*-methyltyramine → 4-*O*-methyldopamine → *iso*-HVA) as we know that tyramine is present in the brain with a particularly high concentration in the hypothalamus and caudate nucleus [28, 29] and that its *meta*-hydroxylation is possible [30–36].

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