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NORMETADRENALINE METABOLISM IN MAN*

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Abstract—Five female subjects were injected with 9·5 μc of DL-normetadrenaline-1-^{14}C (sp.act. 7·5 mc/mmole). The normetadrenaline was injected into the antecubital vein over a 1-min period, and the urine was collected via an indwelling catheter every 10 min for a total of 70 min. Two additional subjects who had been treated with a monoamine oxidase inhibitor (iproniazid, 50 mg t.i.d.) for 9 and 10 days, respectively, were similarly treated. In each urine sample the normetadrenaline and its metabolites were separated by ion exchange and their radioactivity measured by liquid scintillation. Normetadrenaline, which represented 64 ± 3% of the total radioactivity in the first 10-min post-injection period rapidly decreased thereafter. At the same time there was a rapid and marked increase in the 3-methoxy-4-hydroxymandelic acid and the 4-O-sulfate conjugate of normetadrenaline; there was also a moderate increase of 3-methoxy-4-hydroxyphenylglycol sulfate (see Fig. 2). No detectable amounts of 3,4-dihydroxymandelic acid or 3,4-dihydroxyphenylglycol sulfate were found after an injection of normetadrenaline, which indicates that these compounds are not derived from normetadrenaline.

In those subjects who had been previously treated with a monoamine oxidase inhibitor (iproniazid) the deamination of normetadrenaline was inhibited. This was reflected by a decrease in the formation of 3-methoxy-4-hydroxymandelic acid and 3-methoxy-4-hydroxyphenylglycol sulfate and an increase in the normetadrenaline conjugate.

It is now generally believed that in man[1–5] and in animals[6–8] one of the major catabolites of noradrenaline (norepinephrine, arterenol) is 3-O-methylnoradrenaline (normetadrenaline, normetanephrine, and normetarterenol). This catabolite is the O-methylated product of noradrenaline, and its production is dependent largely upon the enzyme O-methyl transferase, which is found in large quantities in the liver and in relatively small amounts in other tissue.[6, 9, 10] The purpose of these experiments is to present new information on the metabolism of normetadrenaline in man and to demonstrate the effect of monoamine oxidase inhibition upon the metabolism of normetadrenaline.

METHOD

Intravenous injection of DL-*normetadrenaline-*1-^{14}*C.* Five female subjects beween the ages of 20 and 25 years were injected with 9·5 μc of DL-normetadrenaline-1-^{14}C (sp. act. 7·5 mc/mmole†). The labeled normetadrenaline was mixed with 10 ml of physiological saline and injected into the antecubital vein over a 1-min period.

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† From Calbiochem, Los Angeles, Calif. Amberlite IRC-50, CG-60, type 2, from Rohm and Haas Co., Philadelphia, Pa. Dowex-1-X2, 200–400 mesh, from Calbiochem, Los Angeles. Iproniazid phosphate (Marsilid), 50-mg tablets, from Hoffman-La Roche, Inc., Nutley, N.J.

Monoamine oxidase inhibition. In addition two subjects who had been previously treated with iproniazid (50 mg t.i.d. for 9 and 10 days respectively) were also intravenously injected with DL-normetadrenaline-1-^{14}C.

Urine collections. Each subject was catheterized. The urines were collected via an indwelling catheter (Foley) 10 min after the beginning of the injection and every 10 min thereafter for a total of 70 min. Urines were also collected for 6 hr and 24 hr on three subjects.

Separation of urinary catabolites of normetadrenaline. A dilution of each urine sample was prepared and its radioactivity measured with a Tri-Carb liquid scintillation spectrometer. The method of assay consisted of placing a 1·0-ml aliquot in each of two 20-dram glass counting vials to each of which was added 15·0 ml of previously prepared scintillation liquid (15 g napthalene, 2 g of 2,5-diphenyloxazole (PPO), and 50 mg 1,4-*bis*-2(phenyloxazolyl)-benzene (POPOP) in 500 ml ethanol and 500 ml toluene).

An aliquot of urine containing 70,000 to 100,000 disintegrations/min (dpm) was placed on a column of Amberlite IRC-50 (1 cm × 5 cm). The column was washed with 30 ml of water. The combined effluent and wash was saved and its radioactivity measured.

The Amberlite column was then eluted with 40 ml of 0·5 N acetic acid. An aliquot of the eluate, containing 500 to 1,500 dpm, was evaporated to about 1 ml and chromatographed for 24 hr on Whatman no. 1 paper with *n*-butanol saturated with HCl as the solvent. After drying, the paper was cut into 1-cm strips. Each strip was placed in a 20-dram counting vial filled with scintillation liquid (4 g of PPO and 100 mg POPOP in 1,000 ml toluene), and its radioactivity was measured in the Tri-Carb spectrometer. Two radioactive peaks were obtained, corresponding to normetadrenaline and an unknown compound. The relative activity of each peak was then determined as a percentage of the total radioactivity recovered in the Amberlite eluate.

An aliquot of the effluent and wash from the Amberlite, containing 30,000 to 40,000 dpm, was placed on a Dowex-1-X2 (200–400 mesh) acetate column (1·0 × 12·0 cm). The column was washed with 100 ml of water and then attached to an autogradient elution system consisting of four series-connected cylinders, each of which contained 275 ml of solution; the first and second of the series contained water, the third, 2 M ammonium acetate buffer, pH 4·8, and the fourth 6 M ammonium acetate buffer, pH 4·8; 5-ml fractions were collected. Alternate fractions were monitored for radioactivity. Radioactive fractions composing a single peak were pooled and their total radioactivity measured. Recovery of the radioactivity placed on the IRC-50 column was 95 ± 5%; recovery from the Dowex-1 column was 94 ± 7%.

The consistency of position of the major metabolites in the Dowex-1 eluate allows an accurate determination of the associated radioactive peaks. The positions of the smaller radioactive peaks which represent the minor metabolites, being widely separated from the major metabolites, are also easily distinguishable.

RESULTS

The basic catabolites are adsorbed and eluted from the Amberlite IRC-50, and the acidic catabolites are adsorbed and eluted from Dowex-1-X2 column. The neutral compounds pass through both the Amberlite and Dowex columns. Figure 1 is a

schematic presentation of how these various catabolites are formed from noradrenaline via normetadrenaline.

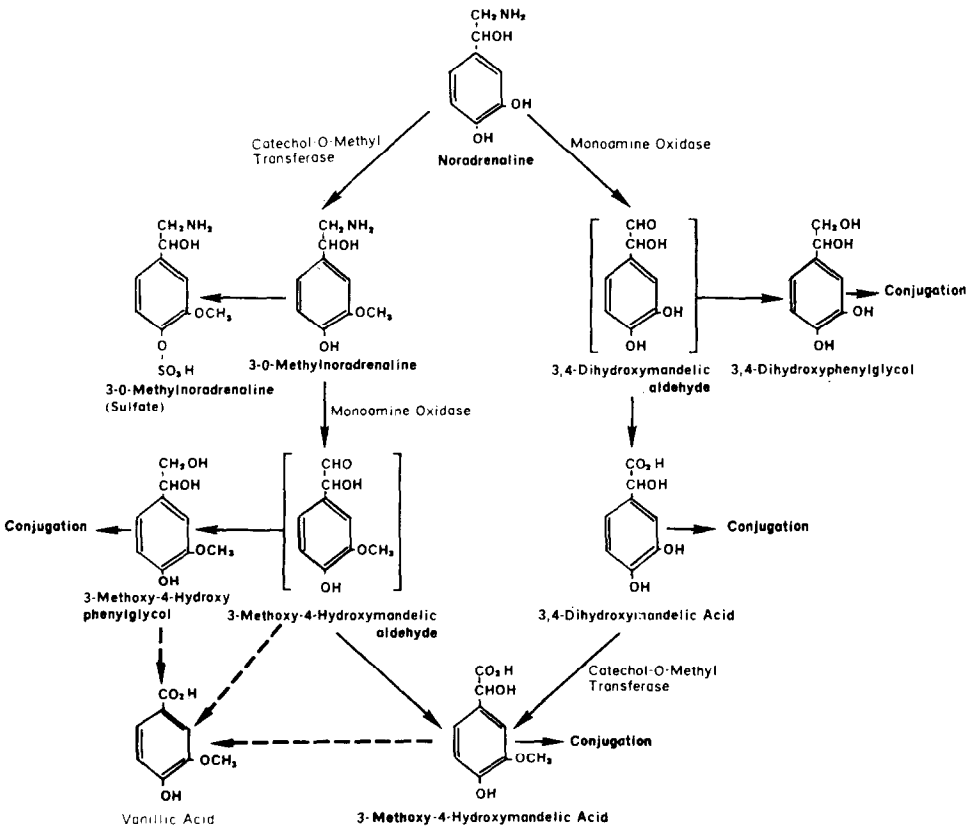

FIG. 1. Alternative pathways for the metabolism of noradrenaline indicating the metabolic pattern of 3-O-methylnoradrenaline and its relationship to noradrenaline metabolism.

Basic catabolites

The normetadrenaline catabolites eluted from the IRC-50 fraction are the basic compounds and include normetadrenaline and an unknown. Normetadrenaline represents 64 ± 3% of the total radioactivity recovered in the first 10-min sample. The radioactivity of the normetadrenaline rapidly declined in the subsequent collection periods; i.e. 30 to 40 min post injection the normetadrenaline represents only 3 ± 2% of the total radioactivity and after 24 hr, approximately 1%. Trace amounts of the basic unknown are detectable throughout the collection periods.

Acidic catabolites

The acidic catabolites of normetadrenaline are best described in the order in which they are eluted from the Dowex column:

1. *4-O-Sulfate conjugate of normetadrenaline.* Conjugate I was previously described as a product of noradrenaline metabolism in man.[1,2] In the first 10-min period following an injection of normetadrenaline this catabolite constitutes a small amount of the total radioactivity, i.e. 1 ± 1%, but gradually increases so that in the 20- to 30-min

TABLE 1. EXCRETION PATTERN OF 3-O-METHYLNORADRENALINE AND METABOLITES AT 10-MINUTE INTERVALS DURING A 70-MIN POST-INJECTION PERIOD AND FOR 6 AND 24 HR POST INJECTION*

Subjects T. K. and D. G. were previously treated with a monoamine oxidase inhibitor (iproniazid, 50 mg t.i.d.) for nine and ten days respectively.

Time collected after infusion (min)		IRC-50 Fractions			Dowex-1 fractions							Neutral fraction	Recovered dose
		Nor.†	Normet.	Unknown	Normet. Conj. I	Normet. Conj. II	Unknown 1B	MOMA	Unknown 2	MOPEG SO_4	Unknown 3	Dowex eff. 2	(%)
0-10													
	Normal	0	64 ± 3	3 ± 1	1 ± 1	1 ± 1	1 ± 1	15 ± 5	1 ± 1	1 ± 1	1 ± 1	2 ± 1	8 ± 1
	Subject T. K.	0	79·7	2·8	0·9	0·5	0·5	2·9	0·4	0·4	0·5	1·8	6·9
	Subject D. G.	0	94·1	trace	trace	trace	trace	trace	trace	trace	trace	trace	6·4
10-20													
	Normal	0	15 ± 7	1 ± 1	6 ± 2	2 ± 1	3 ± 2	61 ± 10	1 ± 1	6 ± 4	2 ± 1	2 ± 1	5 ± 2
	T. K.	0	42·9	2·5	16·2	1·0	0·4	17·4	0·5	2·1	1·5	4·6	2·1
	D. G.	0	68·3	2·4	6·2	0·6	0·8	6·8	0·3	3·6	0·5	0·9	6·9
20-30													
	Normal	0	7 ± 4	trace	16 ± 6	2 ± 1	5 ± 3	53 ± 10	2 ± 1	9 ± 2	3 ± 1	3 ± 2	4 ± 1
	T. K.	0	21·3	0·9	35·0	1·0	1·6	23·6	0·8	2·4	2·1	8·6	1·6
	D. G.	0	24·2	1·0	34·8	0·3	3·0	26·6	0·7	2·1	2·5	2·7	1·4
30-40													
	Normal	0	3 ± 2	trace	16 ± 4	2 ± 1	5 ± 3	61 ± 11	1 ± 1	7 ± 2	2 ± 1	2 ± 1	3 ± 1
	T. K.	0	9·6	0·9	49·2	trace	2·7	25·4	1·0	4·2	2·8	2·3	1·3
	D. G.	0	13·3	0·6	41·3	1·6	2·1	26·6	1·3	2·9	2·6	6·4	1·6
40-50													
	Normal	0	3 ± 2	trace	26 ± 9	1 ± 1	6 ± 3	51 ± 14	1 ± 1	7 ± 2	2 ± 1	2 ± 1	3 ± 1
	T. K.	0	7·7	0·5	56·5	trace	3·3	21·7	1·8	3·4	3·8	1·2	1·8
	D. G.	0	7·9	0·6	50·3	1·4	3·7	26·2	0·7	3·2	1·5	3·2	1·4

TABLE 1.—*continued*

50–60												
	Normal	0	2 ± 1	trace	27 ± 10	2 ± 1	6 ± 2	49 ± 10	3 ± 1	6 ± 3	2 ± 1	2 ± 1	3 ± 1
	T. K.	0	7·3	0·4	57·3	1·6	3·3	19·7	trace	4·3	4·1	1·1	1·3
	D. G.	0	6·6	0·5	52·8	2·8	2·6	26·1	0·9	3·7	1·8	2·1	1·3
60–70													
	Normal	0	2 ± 1	trace	24 ± 9	4 ± 1	8 ± 2	44 ± 11	2 ± 1	9 ± 2	3 ± 1	3 ± 2	2 ± 1
	T. K.	0	5·8	0·3	57·6	1·8	3·2	17·5	2·9	3·7	3·8	2·1	1·5
	D. G.	0	5·3	0·7	52·9	3·9	3·7	25·4	2·1	1·7	1·0	3·1	2·7
						Pooled Samples							
0–70 Min													
	Normal	0	21·1	1·1	10·1	1·4	3·7	48·5	1·1	5·0	1·7	3·4	29·1
	T. K.	0	42·1	1·8	25·5	0·7	1·5	13·2	9·8	2·1	1·9	2·8	16·5
	D. G.	0	54·4	1·0	19·7	1·1	1·5	12·1	0·7	2·3	0·9	1·7	21·3
0–6 hr													
	Normal	0	10·4	0·6	21·8	2·3	7·6	42·6	1·3	6·4	2·2	3·3	65·3
	T. K.	0	20·5	0·5	60·3	0·7	2·6	8·1	1·2	3·1	1·7	2·3	49·0
	D. G.	0	22·7	0·7	48·7	0·9	3·0	13·8	0·5	4·4	1·2	2·4	56·4
0–24 h													
	Normal	0	8·7	0·6	27·6	2·4	7·6	38·3	1·2	6·8	1·4	3·5	79·8
	T. K.	0	12·6	0·3	66·5	0·7	2·8	7·3	1·2	3·4	1·5	2·6	75·3
	D. G.	0	17·5	0·5	54·0	1·1	3·1	13·0	0·6	4·6	1·4	2·7	75·5

* All data are expressed as percentage of recovered radioactivity ± SD.

† Nor., noradrenaline; normet., 3-O-methylnoradrenaline or normetadrenaline; normet. conj. I, 3-O-methylnoradrenaline sulfate; normet. conj. II is unidentified; MOMA, 3-methoxy-4-hydroxymandelic acid; MOPEG SO_4, 3-methoxy-4-hydroxyphenylglycol sulfate; Dowex eff., Dowex effluent or neutral fraction.

post-injection period it represents 16 ± 6%, and 60 to 70 min post injection it represents 24 ± 9% (see Table 1 and Fig. 2). The amount of the conjugate continues to rise so that in the 0 to 6-hr pooled sample it represents 21·8%, and in a 24-hr pooled sample it represents 27·6% of the total radioactivity.

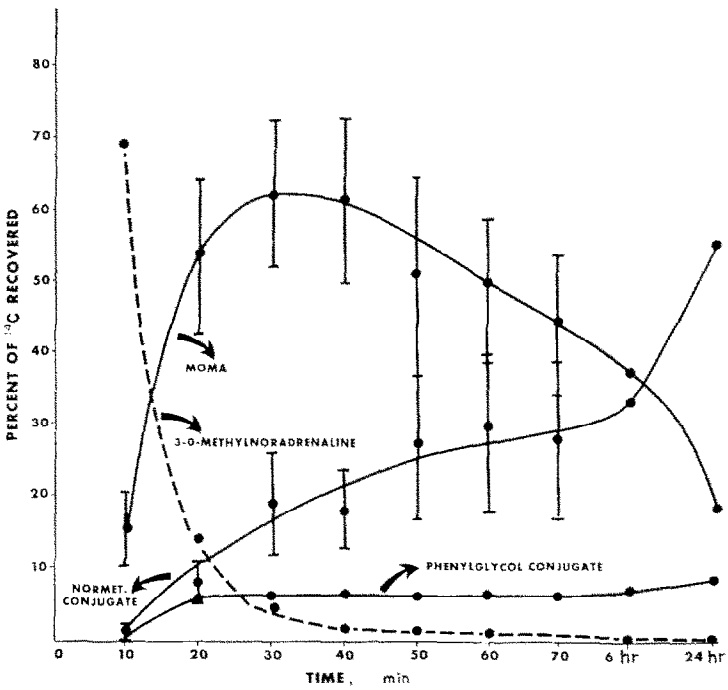

FIG. 2. A comparative excretion pattern showing the rapid decrease in radioactive 3-O-methylnoradrenaline and a concomitant rise in 3-methoxy-4-hydroxymandelic acid, 3-O-methylnoradrenaline conjugate, and 3-methoxy-4-hydroxyphenylglycol conjugate.

2. *Normetadrenaline conjugate II.* This fraction is a conjugate which has not been definitely identified. It is also a catabolite of noradrenaline. In these experiments it represents a small amount of the total radioactivity, i.e. trace to 4 ± 1%, and does not change greatly from one collection period to another (see Table 1).

This peak presumably contains the glucuronic acid conjugate of normetadrenaline. After treatment of the IRC-50 effluent with β-glucuronidase (bacterial type 2 from Sigma Chemical Co.), at pH 5·5 for 24 hr at 37°, most of the radioactivity normally found in this peak may be recovered as free normetadrenaline. After incubation of the dowex peak (conjugate II) under the same conditions, only 10 to 20% of the radioactivity may be recovered as normetadrenaline. However, increasing the glucuronidase activity results in an increase in the recovery of normetadrenaline.

3. *Unknown compound, peak 1B.* This fraction represents an unidentified metabolite of normetadrenaline. It is also found as a product of noradrenaline metabolism[2, 3] and is clearly distinguishable from other metabolic products. The radioactivity in this fraction moves as a single compound when chromatographed in either *n*-butanol with N HCl or isopropanol–ammonia–water (4 : 1 : 1). It is not a major metabolite since, in terms of total radioactivity, the fraction increases only from 1 ± 1% in the

first 10 min post injection to 6 ± 2% in the 50- to 60-min post-injection period (see Table 1).

4. 3-*Methoxy*-4-*hydroxymandelic acid* (*MOMA*). This metabolite was first identified in the urine by Armstrong *et al.*[11] and represents a major metabolic product of both adrenaline and noradrenaline metabolism. MOMA represents 61 ± 10% of the total radioactivity recovered in the 10- to 20-minurine fraction; subsequently the percentage of the radioactivity of MOMA gradually decreases so that by the 70-min post-injection period it represents 44 ± 11% and with the 24-hr pooled sample, 38·3% of the total recovered radioactivity (see Table 1 and Fig. 2).

5. 3,4-*Dihydroxymandelic acid* (*DOMA*). No detectable amounts of this compound were found. It normally occurs in the fraction marked Unknown 2 (Table 1).

6. *Vanillic acid.* Whereas one would expect vanillic acid to be a metabolic product of normetadrenaline,[12, 13] it is in this instance impossible to detect because the labeling in the normetadrenaline (1-^{14}C) is on a carbon which is not retained in the formation of vanillic acid (Fig. 1). It normally occurs in the fraction marked unknown 2 (Table 1).

7. *Sulfate of* 3-*methoxy*-4-*hydroxyphenylglycol* (*MOPEG sulfate*). This compound has been previously identified by Axelrod *et al.*[14] as a catabolite of adrenaline–noradrenaline metabolism. It is quite natural that it should also be a metabolite of normetadrenaline. In terms of total radioactivity, it gradually increases from approximately 1 ± 1% in the first 10 min to 9 ± 2% in the 60- to 70-min post-injection period; excretion continues at approximately 7% for 24 hr (Table 1).

8. *Sulfate of* 3,4-*dihydroxyphenylglycol.* No detectable amounts of this were found. It normally occurs in the fraction marked unknown 3 (Table 1).

9. *Column residue.* There is very little radioactivity retained on the column, thereby implying that in terms of radioactivity, there were only small amounts of other metabolic products.

Dowex effluent (*neutral catabolites*)

From previous work[3] it would seem that a large percentage of the Dowex effluent is the glycol of normetadrenaline. Nevertheless the amount in terms of total radioactivity is quite small, since the Dowex effluent at any single 10-min period never exceeded 3 ± 2% and therefore could hardly be considered important.

Chromatography of this fraction in *n*-butanol–acetic acid–water (4 : 1 : 5) indicates two slow-moving peaks (R_f less than 0·15) and one fast-moving peak (R_f 0·55 to 0·60). The faster peak corresponds to 3-methoxy-4-hydroxyphenylglycol and amounts to about one half the radioactivity of this peak.

Monoamine oxidase inhibition

Two subjects who had received iproniazid (50 mg t.i.d.) for 9 and 10 days, respectively, were also injected intravenously with normetadrenaline and their metabolic pattern compared to that of normal subjects. Since iproniazid is a known monoamine oxidase inhibitor, it seems that the conversion of normetadrenaline to 3-methoxy 4-hydroxymandelic acid would be inhibited (Fig. 1). From the results in Subjects T. K. and D. G. (see Table 1) it is quite clear that this does happen; there is a very large decrease in the formation of MOMA. For example, in the 10- to 20-min post-injection period there is a decrease from 61 ± 10% total radioactivity to 17·4% in Subject

T. K. and 6·8% in Subject D. G. This decrease in MOMA continued throughout the collection periods.

As might also be expected, there was a significant decrease in the 3-methoxy-4-hydroxyphenylglycol sulfate. Further, the unknown peak 1 B showed a decrease similar to that of 3-methoxy-4-hydroxyphenylglycol sulfate, from which it may be inferred that under normal metabolic conditions this unknown is deaminated.

Concomitant with this decrease in MOMA, there was a marked and rapid increase in the normetadrenaline sulfate, which became apparent in the 20- to 30-min post-injection period and continued, so that by the 60- to 70-min period it represented 57·6% in Subject T. K. and 52·9% in D. G. The normal for a similar period is 24 ± 9% (Table 1).

From these results it seems likely that iproniazid tends to decrease the rate at which normetadrenaline is metabolized, since the amount of normetadrenaline in terms of total radioactivity is greater in all periods and especially in the first 10 min post injection. For example, in Subject D. G. the total amount of radioactivity represented by normetadrenaline was 94·1% in the first 10 min post injection and 68·3% in the second 10 min as compared in normal subjects to respective figures of 64 ± 3% and 15 ± 7% (see Table 1).

DISCUSSION

The fact that normetadrenaline is normally found in human urine[1–6, 15] seems to indicate that it is released into the blood stream and cleared by the kidney, thereby implying at least some similarity in the disposition of circulating endogenous catabolite and the intravenously injected catabolite. Further, it has been shown that 'low turnover' pheochromocytomas are capable of synthesizing large quantities of normetadrenaline,[16] and several investigators have demonstrated increased amounts of normetadrenaline in the urine of pheochromocytoma patients.[5, 16] The experiments herein described indicate that when normetadrenaline is intravenously injected, it appears almost immediately in the urine in large amounts but rapidly decreases (see Fig. 2). Concomitant with this rapid decline is an increase in normetadrenaline conjugate and the deaminated metabolite, MOMA; the presence of the latter emphasizes the role of deamination. Contrary to the rabbit experiments of de Potter *et al.*[17] no demethylation was noted in the human.

Whereas a number of investigators[10, 18–22] have demonstrated the effect of monoamine oxidase inhibition on the metabolism of noradrenaline, hitherto there have been no experiments on the effect of monoamine oxidase inhibition on normetadrenaline metabolism in man. From these experiments on human subjects, it is quite clear that by inhibiting monoamine oxidase activity, deamination of normetadrenaline is also impeded. This is demonstrated by the fact that after giving iproniazid to normal subjects there is a decrease in the formation of MOMA and 3-methoxy-4-hydroxyphenylglycol sulfate and an increase in the normetadrenaline conjugate (Table 1). Somewhat similar results were obtained from cirrhotic patients in whom the hepatic monoamine oxidase activity was apparently decreased (paper in preparation); it is well established that normetadrenaline is a better substrate for monoamine oxidase than is noradrenaline.[23]

The fact that no significant quantities of DOMA or 3,4-dihydroxyphenylglycol sulfate were found after the injection of normetadrenaline-1-^{14}C in normal subjects

indicates that these compounds are not derived from the metabolism of normetadrenaline and presumably could not be formed from the metabolism of metadrenaline (see Fig. 1). On the other hand, they must come from the precursor of normetadrenaline and metadrenaline—that is, noradrenaline and adrenaline.

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