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DEGRADATION OF NORMETANEPHRINE AND NORPARANEPHRINE IN HUMAN BLOOD

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Abstract—Normeta- and norparanephrine disappear at similar rates when incubated with human blood. The simultaneous appearance of noradrenaline demonstrates that at least a part of this degradation is caused by *O*-demethylation.

Human blood seems to contain one or several enzymatic system(s) able to degrade ortho-methoxy phenols specifically.

BALDESSARINI and Bell[1] and, more recently, Cohn, Dunner and Axelrod[2] demonstrated the presence of catechol-*O*-methyltransferase activity in human blood. To our knowledge, however, the study of catecholamine metabolism in this tissue received little attention, although blood is very easily available from a great number of human subjects.

Recently, enzymatic systems which catalyze the *O*-methylation of noradrenaline and the degradation of normeta- and norparanephrine have been described in rat blood.[3] The formation of noradrenaline simultaneously to the disappearance of normetanephrine indicated an *O*-demethylation reaction.

In this paper, results are presented on the enzymatic destruction of normetanephrine and norparanephrine in human blood.

MATERIALS AND METHODS

Blood samples were obtained from subjects in our laboratory or from healthy subjects accompanying patients receiving treatment at the "Institut de Recherches cliniques", Montreal. Sodium oxalate or heparin were used as anticoagulants. No difference was observed between the two compounds.

S-adenosylmethionine was purchased from PαL Biochemicals, 3,4-dimethoxy-β-phenylethylamine and 4-methoxy-β-phenylethylamine from Calbiochem; 3,4-dimethoxy-β-phenylethanolamine and *N*-methyl, 3,4-dimethoxy-β-phenylethanolamine were gifts from Dr. W. G. Verly. [7-^{14}C]normetanephrine and [7-^{14}C]norparanephrine (40 mc/m-mole) were prepared according to Frère and Verly.[4]

All incubations were performed at 37°.

Recovery of norparanephrine, normetanephrine, metanephrine and 3,4-dimethoxy-β-phenyl-ethanolamine after incubation was estimated as follows: the amines were oxidized by periodate to the corresponding aldehydes (norparanephrine yields

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isovanillin, metanephrine and normetanephrine, isovanillin and 3,4-dimethoxy-β-phenylethanolamine, veratraldehyde). The aldehydes were extracted by benzene and separated by gas–liquid chromatography.[4] A crude preparation of rat liver catechol-*O*-methyltransferase was obtained by ammonium sulfate precipitation.[5,6] This preparation was devoid of any normeta- or norparanephrine degrading activity.[6] Unless indicated otherwise, all the blood samples of a given experiment were aliquots derived from one sample obtained from one subject. The errors (one standard deviation) were computed as in refs. 4 and 6.

RESULTS

(a) *Destruction of norpara- and normetanephrine incubated in blood*

A mixture of [^{14}C]normeta- and [^{14}C]norparanephrine was incubated in 2 ml blood from four subjects. The results of Table 1 show the recovery of normeta- and norparanephrine after 10 min of incubation compared with a standard in which blood was replaced by water.

TABLE 1. RECOVERY OF NORMETA- AND NORPARANEPHRINE AFTER INCUBATION IN BLOOD

Subject no.	Recovery (%)	
	Normetanephrine	Norparanephrine
1	Undetectable	Undetectable
2	7 ± 2	8 ± 2
3	11 ± 2	11 ± 2
4	50 ± 5	50 ± 5
Reference: Water	100 ± 5	100 ± 5

Two ml of blood were incubated for 10 min with 120 μmoles phosphate buffer, pH 7·8, 0·56 μmole cystein, 0·01 μc (0·250 nmole) [^{14}C]normetanephrine and 0·006 μc (0·150 nmole) [^{14}C]norparanephrine (total volume: 3·0 ml). The reference sample contained 2·0 ml of water instead of blood. A recovery of 2 per cent or less was undetectable.

(b) *Identification of noradrenaline as a degradation product*

[7-^{14}C]Normetanephrine (0·65 μc) were incubated with 5 ml blood and 0·56 μmole cysteine. After 120 min, 1 mg unlabelled noradrenaline bitartrate hydrate was added as carrier and the reaction was terminated by addition of 3·5 ml 7·5 % trichloracetic acid. The supernatant was collected, adjusted to pH 8·4 with a saturated solution of Na_2CO_3 and was percolated through a column of activated alumina (10 × 2 cm), prepared according to Masuoka and Brunjes.[7,8] The column was washed with water and eluted with 0·2 N acetic acid. A 0·1-ml aliquot of the acid eluate (total volume, 20 ml) was chromatographed on paper in the system described by Crawford.[9] A radioactive peak representing about 7·0 nc was found to be associated with the noradrenaline spot as revealed by ninhydrin.

The degradation product (or one of the degradation products) was also found to be susceptible to *O*-methylation by rat liver catechol-*O*-methyltransferase, under adequate conditions: two samples were incubated in a total volume of 3·0 ml containing 120 μmoles phosphate buffer, pH 7·8, 0·56 μmoles cysteine. One ml blood, 0·01 μc (0·25 nmole) of each [7-^{14}C]normeta- and [7-^{14}C]norparanephrine (final pH, 7·4). The second sample also contained 2·0 μmoles S-adenosyl-L-methionine, 50 μmoles $MgCl_2$ and 20 mg of a crude preparation of rat liver catechol-*O*-methyl transferase. After 7 min of incubation, recovery of normeta- and norparanephrine was as follows:

	Normetanephrine	*Norparanephrine*
Sample I:	38 ± 3%	38 ± 3%
Sample II:	56 ± 4%	37 ± 3%

This result can be explained if one assumes that, in both samples, normeta- and norparanephrine were *O*-demethylated to noradrenaline; in the second sample, the important amount of catechol-*O*-methyltransferase could then catalyze the remethylation of enzymatically produced noradrenaline. At this pH, rat liver catechol-*O*-methyltransferase produced 93 per cent of normetanephrine and 7 per cent norparanephrine[6] but this latter amount was too small to be experimentally detected.

(c) *Properties of the normeta- and norparanephrine degrading system(s)*

(1) *Substrate specificity.* The ability of blood samples to degrade two other *O*-methylated catecholamines (metanephrine and 3,4- dimethoxy-β-phenylethanolamine) was investigated. As above stated, periodate oxidation transforms metanephrine into vanillin and 3,4-dimethoxy-β-phenylethanolamine into veratraldehyde. Three samples were prepared containing 2·5 ml blood, 120 μmoles phosphate buffer, pH 7·8 and 0·56 μmole cysteine (in a total volume of 4·0 ml) and incubated for 120 min. Following incubation, 0·5 mg metanephrine.HCl + 0·5 mg 3,4-dimethoxy-β-phenylethanolamine.HCl were added to sample I; to sample II was added 0·5 mg dimethoxy-β-phenylethanolamine.HCl prior to incubation and 0·5 mg metanephrine.HCl following incubation. To sample III was added 0·5 mg metanephrine.HCl prior to incubation and 0·5 mg dimethoxy-β-phenylethanolamine.HCl following incubation. Each sample thus contained an internal standard.

After separation by gas–liquid chromatography, the areas of the peaks of veratraldehyde and vanillin were measured by planimetry.

The $\frac{\text{veratraldehyde peak area}}{\text{vanillin peak area}}$ ratio was found to be:

—in sample 1: 1·53 ± 0·08
—in sample 2: 1·61 ± 0·08
—in sample 3: 1·93 ± 0·08

The results indicated that no dimethoxy-β-phenylethanolamine disappeared during incubation since the ratio in sample 2 is not lower than that in sample 1. On the other hand, metanephrine disappeared during incubation since the ratio is higher in sample 3 than in samples 1 and 2. The increased ratio indicated that about 20 per cent (100 μg) of the initial metanephrine disappeared.

TABLE 2. INFLUENCE OF METHOXY-AROMATIC COMPOUNDS ON THE DEGRADATION OF NORMETA- AND NORPARANEPHRINE

Tested compounds	Recovery in %		
	Normetanephrine	Norparanephrine	Global
None	43 ± 6	41 ± 6	42 ± 8
Vanillin	68 ± 9	71 ± 10	69 ± 8
Isovanillin	68 ± 9	71 ± 10	69 ± 8
Metanephrine	90 ± 12	78 ± 10	85 ± 8
3,4-Dimethoxy-β-phenylethanolamine	49 ± 7	44 ± 6	47 ± 8
N-Methyl 3,4-dimethoxy-β-phenyl-ethanolamine	47 ± 6	45 ± 6	46 ± 8
3,4-Dimethoxy-β-phenylethylamine	50 ± 7	48 ± 7	49 ± 8
Veratraldehyde	54 ± 7	50 ± 7	52 ± 8
4-Methoxy-β-phenylethylamine	37 ± 5	38 ± 5	37 ± 8
Reference: water instead of blood	100 ± 5	100 ± 5	100 ± 5

0·5 ml of blood, 0·56 μmole cysteine, 0·01 μc (0·25 nmole) [^{14}C]normeta and 0·007 μc (0·175 nmole) [^{14}C]norparanephrine and 24 μmoles phosphate buffer, pH 7·8 were incubated with 1 mg of the tested compound in a total volume of 1·5 ml for 30 min. The reference sample contained 0·5 ml of water instead of blood.

(2) *Influence of other* O*-methylated catechols on the degradation of normeta- and norparanephrine.* Table 2 presents results obtained with various ortho-methoxy-phenols, dimethoxy and monomethoxy-aromatic compounds. Only the first class significantly increased the recovery of normeta- and norparanephrine, and metanephrine was the most efficient.

DISCUSSION

These experiments demonstrate the presence of one or several enzymatic systems which degrade normeta- and norparanephrine in human blood. The activity varies from subject to subject but none of the blood samples tested was found to be devoid of activity. The possible presence of monoamine oxidase in blood cannot explain the disappearance of normetanephrine when this amine is estimated as vanillin after periodate oxidation: the action of monoamine oxidase upon normetanephrine would produce 3-methoxy, 4-hydroxy-mandelic acid, which is also oxidized to vanillin by periodate.[10]

At least part of this degradation was caused by *O*-demethylation, as shown by the appearance of noradrenaline during the incubation. While metanephrine was also a substrate of the degrading system(s), 3,4-dimethoxy-β-phenylethanolamine remained stable when incubated with blood. The degrading system(s) thus appeared to be specific for molecules containing the ortho-methoxy-phenol moiety. The increased recovery of both normeta- and norparanephrine when vanillin, isovanillin and metanephrine were added to the incubation mixture also pointed in this direction. In the case of metanephrine, the increased recovery of normeta- and norparanephrine was probably caused by substrate competition. Vanillin and isovanillin were less efficient: it is not, as yet, possible to decide whether the increase, in these cases, was due to

substrate competition or to competitive inhibition. Systems capable of *O*-demethylating methoxy-phenyl groups have been described.[11–17] However, only the system described here seems to be specific for ortho-methoxy-phenols. In our experiments, normeta- and norparanephrine were degraded at similar rates and none of the compounds tested selectively increased the recovery of one of the amines. It is thus probable that the same system(s) is (are) responsible for the degradation of both compounds. Shulgin, Sargent and Naranjo[18] suggested that 4-methoxy-phenyl groups might be demethylated more rapidly than 3-methoxy-phenyl groups. Our results do not verify this suggestion. Moreover, it must be remembered that 3,4-dimethoxy-phenyl compounds were not degraded and did not increase the recovery of normeta- and norparanephrine.

The psychomimetic activity of 3,4-dimethoxy and 4-methoxy-phenyl compounds[19] cannot thus be explained by an action on the system(s) degrading ortho-methoxy-phenols in blood .

It is possible that different results would be obtained with other tissues: very little is known about *O*-demethylation in man.

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