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O-METHYLATION OF NORADRENALINE AND DEMETHYLATION IN RAT BLOOD

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SUMMARY

Rat blood cells O-methylate noradrenaline to normetanephrine without detectable formation of norparanephrine. In contrast, a partially purified blood enzyme O-methylates the noradrenaline in both the *p*- and *m*-positions; the normetanephrine/norparanephrine ratio (12 ± 3 at pH 7.5) in the reaction products is similar to that obtained with purified rat liver catechol *O*-methyltransferase (*S*-adenosylmethionine: catechol *O*-methyltransferase, EC 2.1.1.6).

Incubation with whole rat blood leads to the disappearance of both normetanephrine and norparanephrine; part of the normetanephrine is demethylated to noradrenaline. The absence of *p*-O-methylated products when noradrenaline is incubated with whole blood can probably be accounted for by a preferential action of the degrading enzymes on norparanephrine.

INTRODUCTION

Noradrenaline metabolism in blood has received little attention. BALDESSARINI AND BELL[1] have shown the presence of catechol *O*-methyltransferase (*S*-adenosylmethionine:catechol *O*-methyltransferase, EC 2.1.1.6) in human blood cells. We report here the results of some investigations with rat blood.

O-methylation of the catechol moiety of noradrenaline was estimated as follows: a rat (Wistar strain, 150–200 g) was killed by decapitation and 5 ml of blood were collected in 5 ml of 1% sodium citrate; 5 ml of the suspension were incubated at 37° together with 0.56 μmole cysteine·HCl, 0.55 μmole *S*-adenosyl-L-methionine·HCl, 100 μmoles $MgCl_2$ (due to the presence of citrate, the concentration in $MgCl_2$ had to be increased to 0.014 M in order to reach the maximum rate of O-methylation), 16.7 nmoles (0.8 μC) [7-^{14}C]noradrenaline (48 mC/mmole, purchased from Schwartz Bioresearch) dissolved in 2 ml 0.12 M sodium phosphate buffer, pH 7.8. The total volume was 7 ml and the final pH was 7.5.

Estimations of the O-methylated products were carried out by a modified periodate oxidation method[2]. After 60 min of incubation, the enzymatic reaction was terminated by the addition of 1.5 ml 15% trichloroacetic acid and the precipitate

centrifuged. The yellowish supernatant was decanted, added to 1 ml 15% tricholoroacetic acid and the precipitate was centrifuged. The resultant, colourless supernatant was made alkaline with 2 ml conc. NH_3 and oxidized with 2 ml 5% sodium periodate (which transforms normetanephrine to vanillin, norparanephrine to isovanillin and 3,4-dimethoxy-β-phenylethanolamine to veratraldehyde). After 30 min, the solution was cooled to 0° and the pH adjusted to 6.5. The solution was then extracted with 20 ml benzene. The radioactivity of the aldehydes in the organic phases indicated a 6–10% yield of O-methylated products. This yield varied from rat to rat. Gas-chromatographic analysis of the organic phase showed that vanillin accounted for at least 98% of the total radioactivity and that radioactive isovanillin and veratraldehyde were not detectable (below 1%). This absence of *p*-O-methylation is in contrast with results obtained under similar conditions with rat liver as enzyme source[3]: incubation of whole liver homogenate yields a normethanephrine/norparanephrine ratio of about 32 and incubation of purified catechol *O*-methyltransferase, a ratio of about 12.

5 ml of blood suspension was centrifuged at 10 000 rev./min for 10 min. The precipitate, resuspended in 3 ml of 0.06 M sodium phosphate buffer, pH 7.8, and the supernatant were incubated with [^{14}C]noradrenaline under the conditions described previously. In 60 min, the suspended precipitate O-methylated 2 nmoles of noradrenaline and the supernatant less than 1% of this amount. This result is in agreement with earlier results obtained with human blood[1].

No important loss of enzymatic activity was observed when the citrate suspension was kept at 4° during several weeks. However, after 4 weeks, most of the O-methylating activity was found in the supernatant after centrifugation. The pH of this supernatant was adjusted to 5.0, the precipitate removed by centrifugation and the new supernatant submitted to $(NH_4)_2SO_4$ fractionation. Proteins precipitating between 30 and 60% saturation were collected by centrifugation and redissolved in 0.001 M phosphate buffer, pH 7.0, dialyzed overnight against the same buffer and lyophilized: this fraction contained 70% of the total O-methylating enzymatic activity. Incubation of the enzyme powder (50 mg) with [^{14}C]noradrenaline and estimation of the O-methylation products under the described conditions yielded a normetanephrine/norparanephrine ratio of 12 $\pm$ 3, which was in close agreement with results obtained with the purified liver catechol *O*-methyltransferase. This result suggests that the enzyme involved in O-methylation of noradrenaline in blood is also the catechol *O*-methyltransferase.

When 0.014 μmole (0.5 μC) of [7-^{14}C]normetanephrine prepared enzymatically[2] were incubated for 30 min with 4 ml blood–citrate, 2 ml 0.12 M sodium phosphate buffer (pH 7.8) and 0.56 μmole cysteine·HCl (final pH 7.5), only 28% of the radioactivity could be recovered as vanillin after periodate oxidation. Addition of catechol *O*-methyltransferase cofactors to the incubation mixture increased the recovery to 44% (Table I). This result suggests the possible presence in blood of a demethylating enzyme: in the presence of $MgCl_2$ and adenosylmethionine, the O-methylating enzyme could remethylate the noradrenaline produced by demethylation of normetanephrine and this would account for the 16% increase in the recovery of normetanephrine. Moreover, the disappearance of normetanephrine is inhibited by metanephrine; this result could be explained by a competition between the two O-methylated amines for the same demethylating enzyme. This enzyme might be identical to the oxidative demethylase described by AXELROD[6] and DALY *et al.*[4] in rat liver micro-

TABLE I

RECOVERY OF NORMETANEPHRINE AFTER 30-min INCUBATION

0.5 μC [^{14}C]normetanephrine were incubated for 30 min with 2 ml 0.12 M phosphate buffer, pH 7.8, 0.56 μmole cysteine·HCl and (A) 4.0 ml water; (B) 4.0 ml whole blood; (C) 4.0 ml water and 50 mg of the purified blood enzyme (lyophilized powder). The undegraded normetanephrine was estimated by the periodate oxidation method[3].

Source of enzyme	*Additions*	*Recovery (%)*
(A) —	—	100
(B) Whole blood	—	28
	Catechol *O*-methyltransferase cofactors*	44
	1 mg metanephrine·HCl	76
(C) 30–60% $(NH_4)_2SO_4$ fraction from blood	—	100

* 100 μmoles $MgCl_2$ and 1.9 μmoles adenosylmethionine.

somes, since incubation with microsome-free enzyme preparation (30–60% $(NH_4)_2SO_4$ fraction prepared from blood; see above) did not yield any measurable disappearance of normetanephrine (Table I).

To demonstrate the formation of noradrenaline when normetanephrine is incubated with blood, the following experiment was carried out: 1 μC of [7-^{3}H]normetanephrine (100 mC/mmole, New England Nuclear) was incubated for 30 min with 2 ml blood suspension and 100 μg cysteine·HCl. The reaction was terminated by the addition of 1 ml 7.5% trichloroacetic acid containing 5 mg of each normetanephrine and noradrenaline. The pH of the supernatant was then adjusted to 8.4 with Na_2CO_3

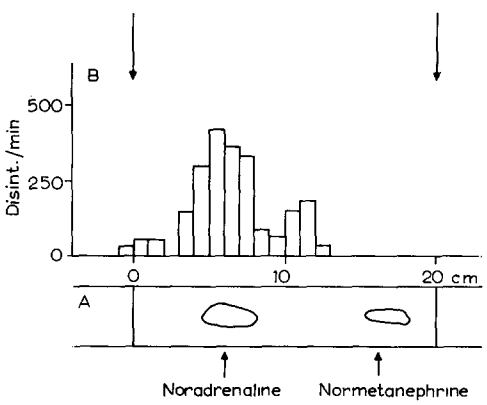

Fig. 1. Identification of noradrenaline by paper chromatography after incubation of [7-^{3}H]normetanephrine with rat blood. 100 μl of the acid eluate (to which unlabelled normetanephrine has been added) from the alumina column were deposited on the starting line (two spots). After 6 h, the chromatography was terminated. One paper strip was sprayed with ninhydrin (A), the other (B) was cut into 1-cm pieces which were eluted with 1 ml of water; to this eluate, 10 ml of scintillating solution (BRAY[11]) were added for counting. Arrows indicate the starting line (0 cm) and the solvent front (20 cm).

and the solution rapidly percolated through an activated alumina column[7,8] which was thereafter washed with water and eluted with 0.2 M acetic acid. After addition of another 2 mg of unlabelled normetanephrine to this eluate (10 ml), a 100-μl aliquot was submitted to ascending paper chromatography (phenol saturated with water in SO_2 atmosphere[9]) showing one main spot of radioactivity with the same R_F as noradrenaline (Fig. 1). By the same procedure, it was shown that rat liver homogenate could also demethylate [^{3}H]normetanephrine into [^{3}H]noradrenaline.

The possible presence of monoamine oxidase in blood cannot explain the apparent disappearance of normetanephrine observed with the periodate method: the action of this enzyme upon normetanephrine would produce 3-methoxy-4-hydroxymandelic acid which is also oxidized to vanillin by periodate[5].

Incubation of a preparation containing [^{14}C]norparanephrine (50% norparanephrine, 11% normetanephrine and 39% unidentified radioactive contaminants[2]), under the same conditions, induced the disappearance of [^{14}C]norparanephrine, identified by gas chromatography as isovanillin after periodate oxidation, at a rate much greater than the chemical degradation described elsewhere[3]. However, the presence of the impurities made it impossible to obtain accurate comparisons between the rates of the disappearance of normetanephrine and norparanephrine.

Our results clearly show the presence of an O-methylating enzyme and of enzymes degrading normetanephrine and norparanephrine in rat blood. The O-methylating enzyme exhibits properties similar to those of liver catechol *O*-methyltransferase. The absence of detectable *p*-O-methylated products when [^{14}C]noradrenaline has been incubated with whole blood can probably be accounted for by a preferential action of the degrading enzymes on norparanephrine. As the *p-O*-methoxyphenyl amine compounds seem to be endowed with some toxic action for the central nervous system[10], their preferential destruction could be physiologically important to correct some noxious result of the catechol *O*-methyltransferase inactivation of catecholamines.

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