The formation of adrenaline from noradrenaline*

It has been demonstrated that the methyl group of methionine can be utilized *in vivo* for the formation of adrenaline[1]. Similarly, adrenaline-^{14}C has been isolated from the adrenal glands of the rat after injection of noradrenaline-^{14}C[2]. The formation of adrenaline in homogenates of dog adrenal glands has been reported[3], but attempts to repeat this work gave negative results[2].

BORSOOK AND DUBNOFF[4] have shown that ATP and methionine are required for the methylation of guanidinoacetic acid; CANTONI[5] has demonstrated that S-adenosylmethionine (AMe) is the active methylating agent. The methylation of noradrenaline to form adrenaline by an enzyme system obtained from bovine adrenal medullae which utilizes either ATP and methionine, or S-adenosylmethionine, is reported here.

After the incubations described below, 1 μmole of unlabeled adrenaline was added to each of the incubation mixtures, and trichloroacetic acid extracts were then prepared. Adrenaline and noradrenaline were separated from each other by ion-exchange chromatography[6] and the radioactivity in the adrenaline fractions was determined with a thin window gas flow counter with an average background of 16 c.p.m.

L-Methionine-methyl-^{14}C was obtained from the Volk Radio-Chemical Company. S-Adenosylmethionine-methyl-^{14}C was prepared as described by CANTONI[7].

Table I shows the distribution of enzyme activity in homogenates of fresh adrenal medullae. The medullae were homogenized in ice cold 0.25 *M* sucrose. After removing the unbroken cells by centrifuging at 1000 $\times$ *g* for 10 min, the homogenate was centrifuged at 20,000 $\times$ *g* for 30 min. The supernatant was decanted and saved for assay. The sediment was washed twice in 0.25 *M* sucrose and finally suspended in a volume of 0.25 *M* sucrose to equal the volume of the 20,000 $\times$ *g* supernatant fraction. It can be seen that with either ATP and methionine, or with S-adenosylmethionine as substrates, essentially all of the enzyme activity is found in the supernatant fraction.

TABLE I

ADRENALINE FORMATION IN ADRENAL MEDULLARY CELL FRACTIONS

Cell fraction	*Methyl donor*	*Adrenaline c.p.m.*
1. 0.5 ml supernatant + 0.5 ml particles	Methionine	1140
2. 0.5 ml particles	Methionine	40
3. 0.5 ml supernatant	Methionine	1600
4. 0.5 ml particles	S-Adenosylmethionine	70
5. 0.5 ml supernatant	S-Adenosylmethionine	3530

To each reaction mixture was added $MgCl_2$, 200 μmoles; reduced glutathione, 2.5 mg; *l*-noradrenaline, 1 μmole; and 0.2 ml tris buffer, pH 7.4, 0.5 *M*. Nos. 1, 2 and 3 contained 10 μmoles ATP and 1 μmole L-methionine-methyl-^{14}C ($3.1 \cdot 10^5$ c.p.m.). Nos. 4 and 5 contained 0.36 μmole S-adenosylmethionine-methyl-^{14}C ($5 \cdot 10^4$ c.p.m.). The total volume was 1.8 ml. Incubations were for 2 h at 37° under nitrogen.

Table II presents data on the $(NH_4)_2SO_4$ fractionation of the enzymes. Adrenal medullae were homogenized in 2.5 volumes of cold 0.1 *M* sodium acetate, pH 5.6. The homogenate was centrifuged at 20,000 $\times$ *g* for 30 min. The supernatant was decanted through glass wool and fractionated by adding the calculated volumes of saturated $(NH_4)_2SO_4$ solution. Prior to use, each of the enzyme preparations was dialyzed against 0.1 *M* sodium acetate, pH 6.6, for 4 h. All operations were performed at 2°. Both the methionine activating enzyme (MAE) and noradrenaline methylpherase (NMP) are found in the 0.35 to 0.50 saturated $(NH_4)_2SO_4$ fractions.

Comparing the amounts of radioactivity in the adrenaline fractions when ATP and methionine are used as co-substrates to the amounts found when AMe is used as co-substrate, and considering that the initial amount of radioactivity in the AMe was one tenth of the amount of radioactivity in the methionine, it is seen that AMe is utilized at about 20 to 30 times the rate of ATP and methionine for the methylation of noradrenaline.

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TABLE II

AMMONIUM SULFATE FRACTIONATION OF NORADRENALINE METHYLPHERASE

	% $(NH_4)_2SO_4$ saturation	C.p.m./μmole adrenaline/mg protein		% Total activity	
		ATP + Methionine	AMe	ATP + Methionine	AMe
1.	0	1220	2540	100	100
2.	0–35	410	1110	7	8
3.	35–50	1610	4640	31	38
4.	50–75	745	2040	4	5

Each reaction mixture contained reduced glutathione, 2.5 mg; $MgCl_2$, 200 μmoles; *l*-noradrenaline, 1 μmole; 0.2 ml tris buffer 0.5 *M*, pH 7.5; and either 0.14 μmole S-adenosylmethionine-methyl-^{14}C ($2.7 \cdot 10^4$ c.p.m.) or 10 μmoles ATP and 1.0 μmole L-methionine-methyl-^{14}C ($3.1 \cdot 10^5$ c.p.m.). Each incubated 2 h at 37° under nitrogen. 1, 2, 3 and 4 contained, respectively, 2.0, 2.8, 3.0, and 2.3 mg of protein/ml; the total volume in each instance was 1.0 ml.

Table III shows the absolute requirement for ATP and Mg^{++} when methionine is used as the methyl donor. CANTONI[5] has reported these requirements for the MAE and also reported a requirement for glutathione or other –SH compounds for optimal activity of MAE. When AMe is the methyl donor, glutathione is required for optimal activity; Mg^{++} also appears to be necessary. CANTONI AND VIGNOS[8] reported a requirement for –SH compounds for optimal activity of guanidinoacetate methylpherase, but Mg^{++} was not required.

TABLE III

REQUIREMENTS FOR ADRENALINE FORMATION

Reaction medium	C.p.m./μmole adrenaline/mg protein	
	Methionine	AMe
Complete system	1610	4640
No glutathione	1510	2870
No Mg^{++}	0	3370
No ATP	0	
No noradrenaline	200	170

The complete system contained $MgCl_2$, 200 μmoles; reduced glutathione 2.5 mg; *l*-noradrenaline, 1 μmole; 0.2 ml tris buffer, pH 7.4, 0.5 *M*; ATP, 10 μmoles when methionine was used as co-substrate; and either 1 μmole L-methionine-methyl-^{14}C ($3.1 \cdot 10^5$ c.p.m.) or 0.14 μmole S-adenosyl-methionine-methyl-^{14}C ($2.7 \cdot 10^4$ c.p.m.). Final volume was 1 ml. The protein concentration was 3.0 mg/ml. Incubated 2 h at 37° under nitrogen.

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