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DEMETHYLATION OF METHINDIONE

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Demethylation of methindione was shown to take place in vivo in rats. The process involves the participation of the microsomal NADPH-dependent electron transport system. In experiments in vivo demethylation of methindione takes place rapidly and is accompanied by the partial loss of its anticonvulsant properties.
KEY WORDS: methindione; demethylation; inactivation; microsomes.

To understand the special pharmacological properties of the new anticonvulsant drug methindione (2-methylamino-2-ethylindanedione-1,3 hydrochloride) [1, 2], information on its metabolism is necessary.

The object of this investigation was to study one of the pathways of methindione metabolism, namely its demethylation, and to estimate the velocity of this process.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing 150-230 g. The following preparations of methindione were used: 1) ^{14}C-labeled in the keto group, 2) ^{14}C-labeled in the second position of the indanedione ring, 3) ^{14}C-labeled in the methyl group, and 4) nonradioactive. The preparations were given by mouth. The urine from the experimental animals was collected for 28 h. The level of labeled products was measured with a scintillation counter. To compare the levels of excretion of labeled preparations 1 and 3 with the urine, they were injected in doses of equal radioactivity (14 μCi/kg). Fractionation and determination of methindione and its metabolites were carried out by paper (in a system of n-butanol : glacial acetic acid : water, 4 : 1 : 5), thin-layer (silica gel adsorbent; system of isopropanol : n-butanol : 25% ammonia solution : water, 5 : 10 : 0.3 : 2.5), and gas chromatography, using standards and analytical reagents. In the first case radioactive preparations were used (0.13-0.9 μCi/kg), in the rest unlabeled methindione (300 mg/kg). Methindione and its metabolites were isolated from the urine after its preliminary hydrolysis by β-glucuronidase [9]. The activity of the demethylation enzyme system in the postmitochondrial supernatant and in the microsomes of the liver was determined in vitro from the amount of formaldehyde formed [4]. The microsomal fraction was isolated by the method of Cinti et al. [5]. Protein was determined by Lowry's method [6]. To study the kinetics of the demethylation process the values of K_m and V_{max} were analyzed [3]. The binding of cytochrome P-450 with methindione was recorded on the Specord UV VIS spectrophotometer by the method of Schenkman et al. [7]. In

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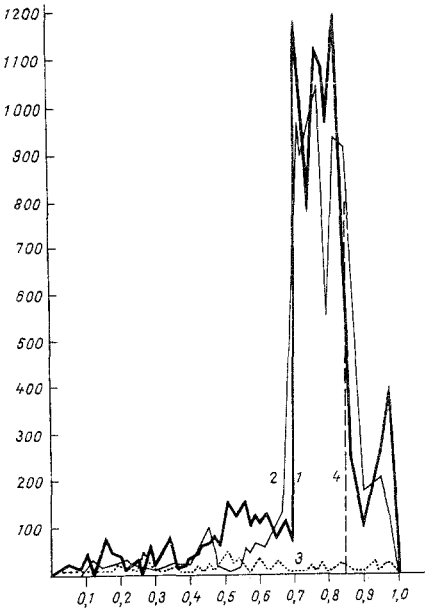

Fig. 1

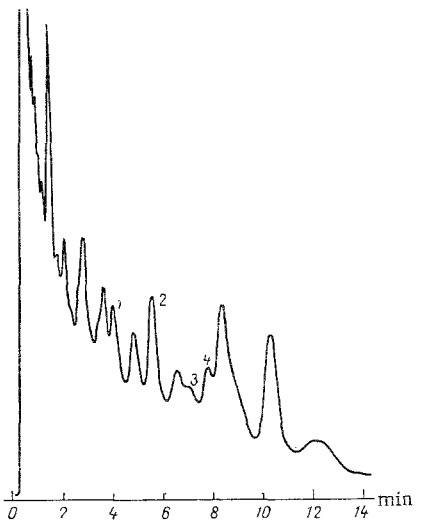

Fig. 2

Fig. 1. Radiochromatographic analysis of 24-h urine of rats receiving methindione. 1, 2, 3) Preparations 1, 2, and 3, respectively; 4) methindione (control). Abscissa, R_f value; ordinate, radioactivity (in cpm per total volume of urine $\times 10^3$).

Fig. 2. Gas-chromatographic fractionation of methindione metabolites isolated from rat urine. Peaks: 2) methindione, 3) 2-amino-2-ethylindanedione-1,3; 1, 4) not identified. Conditions of fractionation: Hewlett-Packard 7620 chromatograph; size of column 2.2 mm $\times$ 300 cm; solid phase Carbowax 20M (5%) on Chromosorb W-AW-DMCS; carrier gas helium; rate of flow of carrier gas 50 ml/min. Temperature of column and vaporizer 200°C.

a separate series of experiments the formaldehyde content was determined in the postmitochondrial supernatant of the rats' livers and in the blood serum in vivo after oral administration of methindione (300 and 800 mg/kg, respectively). Anticonvulsant activity was studied in experiments on C57BL/6 mice by the maximal electric shock method [8]. The ED_{50} value was estimated 30 min after intraperitoneal injection of methindione.

EXPERIMENTAL RESULTS

After administration of preparations 1 and 3 the quantity of radioactive products excreted with the urine was 92.5% and 85.% respectively, of the injected dose. Since preparation 1 is labeled in the stable part of the methindione molecule and preparation 3 in the methyl group, it can only be suggested that removal of the methyl group takes place in vivo in the rat, followed by its oxidation to carbon dioxide, which is eliminated through the lungs. That is evidently why negligible radioactivity was found in the urine after administration of preparation 3.

To confirm the suspected demethylation of methindione a radiochromatographic analysis was made of the 24-h sample of urine from rats receiving preparations 1-3 (Fig. 1). It will be seen in Fig. 1 that the highest radioactivity on the chromatogram in experiments with preparations 1 and 2 was found in the region of R_f values close to that of methindione itself. Preparation 3 had no marked radioactivity at any R_f value, indicating removal of the ^{14}C-methyl group.

Analysis of the urine by thin-layer chromatography revealed the structure of one compound with $R_f = 0.78$. The spot discovered corresponded to the spot containing the standard compound 2-amino-2-ethylindanedione-1,3. Both the standard itself and the compound with $R_f = 0.78$ gave the characteristic reaction with ninhydrin for a primary amino group.

To obtain direct evidence of demethylation of methindione gas-chromatographic analysis of the urine was carried out (Fig. 2). Four compounds were found in the urine, one of which was methindione (peak 2), another 2-amino-2-ethylindanedione-1,3 (peak 3), whereas the other two (peaks 1 and 4) were unidentified metabolites.

TABLE 1. Activity of Methindione Demethylase in vitro

Test object	Concentration of methindione, μM	Enzyme activity (in μ moles CH_2O/g protein/20 min)	
		in presence of NADPH	in absence of NADPH
Postmitochondrial supernatant	0,25	1,61±0,11 (7)	—
	0,75	3,60±0,11 (8)	—
	1,25	4,38±0,26 (5)	0,55±0,08 (5)
	2,5	5,60±0,31 (5)	—
Microsomal fraction	1,25	5,17±0,25 (5)	0 (3)

Legend. Number of experiments in parentheses.

To determine the enzyme systems participating in the demethylation of methindione in vitro its spectrum from the liver microsomes was investigated. The results showed that methindione binds with cytochrome P-450 to form a type 1 differential spectrum. The formation of demethylation products takes place in the microsomal fraction just as in the postmitochondrial supernatant on the addition of methindione and NADPH (Table 1). This fact, together with the ability of methindione to form a complex with cytochrome P-450, is evidence that demethylation is brought about by the NADPH-dependent electron transport system of the microsomes.

The degree of demethylation of methindione in the postmitochondrial supernatant of the rat liver rises proportionally with the increase in concentration of the preparation. The Michaelis–Menten constant was $2 \cdot 10^{-3}$ M, indicating relatively high affinity of methindione for the enzyme. The V_{max} value under the experimental conditions used was 11.1 μmoles CH_2O/g protein/20 min.

Investigations of the demethylation of methindione in vivo showed that the process takes place at relatively high velocity. If the velocity of this process is deduced from formaldehyde formation, both in the liver and in the blood serum it must take place within 3 h. In reality, however, the velocity of demethylation is evidently a little higher, for the formaldehyde revealed by analysis in biological media may be due partly to a methyl group migrating from methindione to the endogenous acceptors.

To assess the role of demethylation of methindione in its anticonvulsant effect the ED_{50} values were compared by the maximal electric shock test for methindione and 2-amino-2-ethylindanedione-1,3. For the first substance ED_{50} was found to be 24 (19-30.2) mg/kg, and 60 (47.2-76.2) mg/kg for the second. Demethylation of methindione can thus be regarded as one of its inactivation processes, which determine the duration of action of the compound in the body.

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