EFFECT OF o,p'-DICHLORODIPHENYLDICHLOROETHANE ON GLUTATHIONE REDUCTASE ACTIVITY AND CONTENT OF SH GROUPS IN THE DOG ADRENALS

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The effect of o,p'-dichlorodiphenyldichloroethane (DDD), a compound inducing atrophy of the adrenal coretx and blocking steroid production, on glutathione reductase activity was studied. As a result of feeding dogs with DDD in a dose of 50 mg/kg body weight for 14 days activation of glutathione reductase took place in the homogenate, cytoplasmic fraction, and disintegrated mitochondria of the adrenals. On the addition of DDD in vitro the activity of the enzyme was unchanged. The content of SH groups expressed per 100 mg tissue was reduced in the homogenate and in all subcellular fractions. If expressed per 100 mg protein the level of SH groups was lowered only in the microsomes and the disintegrated mitochondria.

KEY WORDS: adrenals; glutathione reductase in subcellular fractions; proteins – SH groups; o,p'-dichlorodiphenyldichloroethane.

The adrenals have a high content of glutathione but its role in the biochemical processes taking place in these glands is not yet clear. The hypothesis that glutathione participates in the reactions of steroid formation has not been confirmed [10]. Glutathione is known to regulate the interconversions of microheterogeneous forms of glucose-6-phosphate dehydrogenase (GPD) in the liver [12]. The glutathione–glutathione reductase (E.C. 1.6.4.2) system can evidently participate indirectly in the preservation of the tertiary configuration of GPD, a matter of great importance in the mechanism of steroid production. Glutathione reductase activity in the adrenals is increased by the influence of adrenocorticotropic hormone [11] and reduced by hypophysectomy [8]. On the administration of o,p'-dichlorodiphenyldichloroethane (DDD) to dogs the functional activity of the adrenals is depressed, and this is accompanied by inhibition of GPD activity [1, 6].

The object of this investigation was to determine glutathione reductase activity and the content of SH groups in the adrenal tissue of dogs after receiving DDD.

EXPERIMENTAL METHOD

Male dogs were fed with DDD in a dose of 50 mg/kg body weight daily for 14 days. The method of preparing the homogenate and of isolating the subcellular fractions of the adrenals was described earlier [5]. Parallel investigations were made of the adrenals of experimental and intact dogs. Glutathione reductase was determined spectrophotometrically from the decrease in NADP · H_2 at 340 nm [8] and by amperometric titration on the basis of the increase in glutathione SH groups.

The substrate mixture for the determination of glutathione reductase contained 10^{-3} M oxidized glutathione, 6×10^{-5} M NADP · H_2, and 0.05 M Tris-HCl, pH 7.4. No glutathione was present in the control samples.

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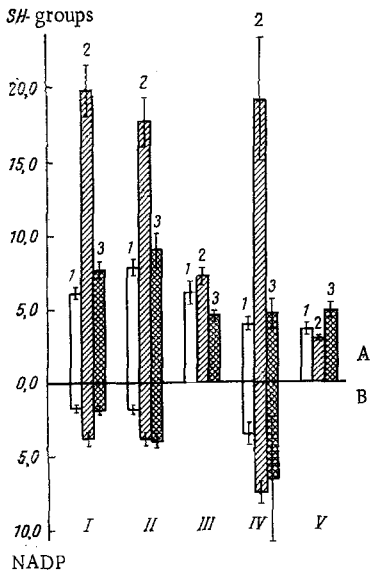

Fig. 1. Effect of DDD on glutathione reductase activity: A) increase in SH groups during incubation for 6 min (in μmoles/100 mg protein); B) decrease in NADP · H_2 in 6 min (in μmoles/100 mg protein); I) homogenate; II) cytoplasm; III) intact mitochondria; IV) disintegrated mitochondria; V) microsomes; 1) control; 2) feeding with DDD; 3) addition of DDD in vitro.

In the experiments in vitro an alcoholic solution of DDD was added to the substrate and control mixtures at the rate of 0.5 mole to 3 ml. The control for these experiments consisted of samples containing an equal volume of alcohol. The change in optical density at 340 nm during the 6 min after addition of the adrenal fractions was recorded with a type EPS-3T (Hitachi) recording spectrophotometer at 26°C. The region of linear decrease in optical density was used to calculate the rate of oxidation of NADP · H_2. The protein content was determined by Lowry's method [9] after preliminary extraction of the lipids and also by Bramhall's method [7]. The SH groups were titrated with 0.001 M $AgNO_3$ in ammonia buffer. A rotating platinum electrode was used.

EXPERIMENTAL RESULTS AND DISCUSSION

The results of the amperometric determination of glutathione reductase showed that the enzyme was present in all fractions of the dog adrenals studied. The highest enzyme activity was found in the cytoplasmic fraction. Similar results were obtained previously by the spectophotometric method [5].

As a result of feeding the dogs with DDD, considerable activation of glutathione reductase took place in the homogenate ($P < 0.001$), the cytoplasmic fraction ($P < 0.01$), and the disintegrated mitochondria (Fig. 1A). Similar results were obtained by determination of the activity of the enzyme on the basis of the decrease in NADP · H_2 ($P < 0.01$, < 0.01, and < 0.05, respectively) (Fig. 1B). DDD did not affect the glutathione reductase activity of the intact mitochondria and microsomes. On the addition of DDD in vitro no significant changes were observed in the activity of the enzyme (Fig. 1).

In the dogs receiving DDD the content of SH groups expressed per 100 mg tissue was reduced in all subcellular fractions of the adrenals except the cytoplasm (Table 1). If the level of SH groups was calculated per 100 mg protein a decrease was observed only in the microsomes and the disintegrated mitochondria. These results indicate changes in the adrenal proteins and, when compared with results obtained for the brain [4], they are evidence of some degree of tissue specificity in the action of DDD. The activation of glutathione reductase observed after feeding with DDD was evidently not the result of direct interaction between DDD and the enzyme. This conclusion is based on the absence of any marked activation of the enzyme by DDD in vitro. In the glutathione reductase reaction the oxidation of 1 mole NADP · H_2 is accompanied by the formation of 2 moles of reduced glutathione. The results given in Fig. 1 show that the formation of SH groups was greater than expected. This was problably the result of repeated reduction of the NADP by endogenous substrates or under the influence of "substrate-free dehydrogenase" [3].

TABLE 1. Distribution of SH Groups in Subcellular Fractions of the Adrenals (M ± m)

Fractions	In μmoles/100 mg wet weight of tissue		P	In μmoles/100 mg protein		P
	control	DDD		control	DDD	
Homogenate	4,64±0,34 (9)	3,72±0,24 (10)	<0,05	6,1±0,44 (17)	5,0±0,39 (12)	>0,05
Cytoplasm	2,80±0,26 (9)	3,14±0,31 (10)	>0,05	7,4±0,20 (17)	7,0±0,60 (12)	>0,05
Intact mitochondria	0,94±0,10 (9)	0,31±0,09 (5)	<0,001	6,0±0,46 (17)	4,0±0,96 (7)	>0,05
Disintegrated mitochondria	0,37±0,06 (9)	0,13±0,01 (5)	<0,001	9,8±0,67 (11)	4,4±1,10 (7)	<0,001
Microsomes	0,57±0,03 (8)	0,42±0,04 (10)	<0,01	7,1±0,45 (17)	4,7±0,59 (11)	<0,01

Note. Number of experiments given in parentheses.

If equilibrium of the glutathione reductase reaction is shifted in the adrenal tissues toward the reduction of glutathione, as it is in other tissues, activation of the enzyme on the addition of DDD will cause a decrease in the NADP · H_2 content in the tissue. The observed inhibition of the reactions of steroid production and, in particular, of 11-β-hydroxylation [2] may thus be the result of activation of glutathione reductase following the NADP · H_2 deficiency in the tissue.

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