EFFECT OF o,p-DICHLORODIPHENYLDICHLOROETHANE AND PERTHANE in vitro ON GLUTATHIONE REDUCTASE ACTIVITY IN THE ADRENALS OF DOGS AND GUINEA PIGS

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o,p'-Dichlorodiphenyldichloroethane (o,p'-DDD) and Perthane, when added in a concentration of 312 μM to homogenate and cytoplasmic fraction of dog adrenals, activate glutathione reductase. In a concentration of 156 μM, o,p'-DDD and Perthane do not affect glutathione reductase activity of the dog adrenals. When given in vitro, o,p'-DDD and Perthane activate glutathione reductase of the guinea pig adrenals. o,p'-DDD has no effect on glutathione reductase activity of the cytoplasmic fraction of dog liver and kidney, thus confirming the high specificity of its effect on the adrenal cortex.

KEY WORDS: glutathione reductase; o,p'-dichlorodiphenyldichloroethane; p,p'-diethyldiphenyldichloroethane (Perthane); adrenals; liver; kidneys.

As a result of the search for the mechanism of action of o,p'-dichlorodiphenyldichloroethane (o,p'-DDD), an inhibitor of adrenocortical function, inhibition of the glucose-6-phosphate dehydrogenase and malic enzyme reactions in the adrenal cortex was discovered [4, 5]. The slowing of these reactions must evidently lead to a deficiency of reduced NADP, an important cofactor in steroid formation. Among the enzymes for which NADP is a cofactor, one which evidently deserves attention is glutathione reductase (EC 1.6.4.2). Feeding dogs with o,p'-DDD leads to glutathione reductase activation in the adrenals [1]. The concrete mechanisms of activation of this enzyme are not clear.

The object of this investigation was to study the possibility of a direct action of o,p'-DDD on adrenal glutathione reductase and also to study the effect of Perthane (p,p'-diethyldiphenyldichloroethane) – an analog of o,p'-DDD – on this enzyme.

EXPERIMENTAL METHOD

The method of preparing the homogenate and the cytoplasmic fraction of dog adrenocortical tissue was described previously [1]. Guinea pig adrenals were homogenized without separation of the medulla.

The incubation mixture for determination of glutathione reductase contained 10^{-3} M oxidized glutathione, $6 \cdot 10^{-5}$ M NADPH, and 0.05 M Tris-HCl buffer, pH 7.4 [7]. A 0.01 M solution of o,p'-DDD or Perthane in ethanol was added to the incubation medium. The alcohol concentration in the medium was 1.5-3.0%. The inhibitors were added immediately before addition of the adrenal preparations. Samples containing an equal quantity of alcohol served as the control.

Glutathione reductase activity was determined spectrophotometrically as the decrease in NADPH at 340 nm. The optical density was recorded by the Hitachi EPS-3T recording spectrophotometer at 30°C for 5 min. To calculate the rate of oxidation of NADPH, the region of linear decrease in optical density was used. Protein was determined by the method of Bramhall et al. [6].

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TABLE 1. Effect of o,p'-DDD and Perthane in vitro on Glutathione Reductase Activity in Dog and Guinea Pig Adrenals (in nmoles NADPH/min/mg protein; M ± m)

Species of animals	Substances added	Homogenate			Cytoplasm		
		ethanol	o,p'-DD	Perthane	ethanol	o,p'-DD	Perthane
Dogs	156 μM of preparations; 0.05 ml of ethanol	10,9±0,6 (10)	11,6±0,8 (12)	9,5±0,6 (10)	14,8±0,4 (9)	15,1±2,1 (11)	16,5±1,7 (9)
	312 μM of preparations; 0.10 ml of ethanol	8,2±0,6[b] (8)	13,6±1,9[a] (11)	10,0±0,6[a] (9)	12,0±0,4[b] (8)	22,8±2,5[a,b] (10)	16,1±1,3[a] (10)
Guinea pigs	156 μM of preparations; 0.05 ml of ethanol	20,5±1,4 (6)	26,0±2,0 (7)	27,3±1,3[a] (6)	34,7±1,2 (8)	38,9±1,4[a] (12)	32,9±1,1 (10)
	312 μM of preparations; 0.10 ml of ethanol	21,0±0,6 (7)	36,5±1,9[a,b] (13)	24,3±1,8 (11)	32,2±0,4 (8)	43,4±2,8[a] (12)	38,3±1,3[a,b] (10)

Legend. 1.a) $P < 0.05$ for comparison of effect of preparations and ethanol; b) $P < 0.05$ for comparison of action of different concentrations of preparation or ethanol. 2) Number of determinations shown in parentheses.

TABLE 2. Effect of o,p'-DDD (312 μM) on Glutathione Reductase Activity in Cytoplasmic Fraction of Certain Dog Tissues (in nmoles NADPH/min/mg protein; M ± m)

Test object	Adrenals	o,p'-DDD
Adrenals	12,0±0,4 (8)	22,8±2,5[a] (10)
Liver	22,8±0,4 (7)	24,3±1,0 (10)
Kidneys	50,6±1,3 (6)	52,1±1,6 (7)

Legend as in Table 1.

EXPERIMENTAL RESULTS

The experimental results indicate that o,p'-DDD and Perthane, in a concentration of 312 μM, activate glutathione reductase in the homogenate and cytoplasmic fraction of the dog adrenals (Table 1). On the addition of o,p'-DDD and Perthane to the homogenate, glutathione reductase activity was increased by 66 and 22% respectively, whereas in the cytoplasmic fraction more marked activation of the enzyme was observed – by 90 and 34% respectively. These results show that o,p'-DDD acts more strongly than glutathione reductase. In the presence of a lower concentration (156 μM) of these diphenyldichloroethane derivatives, glutathione reductase activity remained unchanged.

The experiments thus showed that o,p'-DDD and Perthane can act on glutathione reductase in vitro, but their effect depends on concentration. The absence of significant changes in glutathione reductase activity after the addition of o,p'-DDD observed previously was evidently connected with the low concentration of o,p'-DDD (156 μM) [1].

Glutathione reductase activity in the adrenal tissue (without separation of cortex and medulla) in guinea pigs was twice the level of its activity in the dog adrenal cortex (Table 1).

Both o,p'-DDD and Perthane in vitro activate glutathione reductase in the guinea pig adrenals. By contrast with the adrenals of dogs, in concentrations of 156 μM, o,p'-DDD in the cytoplasmic fraction and Perthane in the homogenate caused an increase in activity of the enzyme.

Activation of glutathione reductase in the adrenals of guinea pigs, in the writers' view, is definitely interesting. We know that in vivo o,p'-DDD and Perthane do not inhibit adrenal function in guinea pigs and do not cause structural lesions in the adrenal cortex [2, 3]. The results now obtained could be evidence that the concept of "resistant species" is to some extent relative.

It is interesting to note that the addition of alcohol to the homogenate and cytoplasmic fraction of dog adrenals caused inhibition of glutathione reductase activity, whereas the glutathione reductase of the guinea pig adrenals was resistant to ethanol.

To study the specificity of the activating effect of o,p'-DDD experiments were carried out with the cytoplasmic fraction of the liver and kidneys (Table 2).

The results are evidence that o,p'-DDD activates the enzyme only in the adrenal. Similar results were obtained by the writers when feeding dogs with o,p'-DDD. These observations confirm the high specificity of action of the inhibitor on the adrenal cortex.

It can be postulated on the basis of these results that the marked activation of the enzyme by o,p'-DDD and Perthane in vitro is a result of the direct interaction between the diphenyldichloroethane derivatives and glutathione reductase, but the mechanism of this activation is not yet clear.

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FREE FATTY ACID CONTENT IN MUSCLES AFTER ADMINISTRATION OF ACTH AND HYDROCORTISONE

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The content of free fatty acids (FFA) in the gastrocnemius muscles 30 min after intraperitoneal injection of 1 unit ACTH/100 g and 1 mg hydrocortisone acetate/100 g body weight was investigated by gas chromatography in experiments on rats. In resting muscles ACTH was shown to increase the stearic acid content whereas hydrocortisone increased the content of both stearic and oleic acids. Changes in the concentration of other FFA were not significant. During a short period of activity involving single regular contractions the stearic acid concentration in the gastrocnemius muscles of intact rats increased. In the experiments with ACTH and hydrocortisone this increase was considerably smaller and was not significant. ACTH and hydrocortisone stimulate the utilization of stearic acid by the muscles during activity.

KEY WORDS: ACTH, hydrocortisone, free fatty acids, muscular contraction.

Hormones of the adenohypophysis and adrenal cortex play a special role in the regulation of lipid metabolism, for significant disturbances of this type of metabolism are observed when they are deficient. However, data on the role of these hormones in the regulation of lipid metabolism are to some degree contradictory. Some workers, for instance, found a decrease in the neutral lipid content in the liver and blood plasma of animals after adrenalectomy [14], whereas others found no such changes [12]. On the other hand, stress has been shown to increase the blood level of free fatty acids (FFA) while at the same time increasing the lipolytic activity of the adipose tissue and liver [7].

Existing data on the effect of ACTH and glucocorticoids on lipolysis are concerned chiefly with adipose tissue and the liver. However, unequivocal data have been obtained only for ACTH. Injection of preparations of this hormone stimulates lipolysis of the adipose tissue and increases the fatty acid concentration in blood plasma [1, 4, 5]. There are few data on glucocorticoids and they point to absence of any effect on lipolysis

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