The Effects of Glutathione on the 11β-hydroxylation of 11-deoxycorticosterone by Bovine Adrenal Cortex Mitochondria

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Contamination of the environment by sulphurous byproducts of human activity can directly and indirectly affect or be harmful to living organisms. Among these effects is the evidence which is accumulating that common sulphur-containing products may influence the biosynthesis of the steroid hormones which to a large extent regulate the reproductive and daily physiological processes of living animals. Since to the best of our knowledge the effects of oxidised or reduced glutathione on corticosteroidogenesis by bovine adrenocortical mitochondria in vitro is not known this investigation was made in the course of a larger study on steroid hydroxylations in these organelles.

Steroid hydroxylation reactions known to take place in bovine adrenal cortex mitochondria include those known to result in the conversion of cholesterol to pregnenolone, the 11β-hydroxylation of 11-deoxycorticosterone to corticosterone and the 18-hydroxylation of corticosterone to 18-hydroxycorticosterone (BRANSOME, 1968). The conversion of cholesterol to pregnenolone (3β-hydroxy-pregn-5-en-20-one) is known to also take place in the mitochondria of other endocrine tissues such as the testis, ovary and placenta and this reaction may well control the biosynthesis of the other steroid hormones. SULIMOVICI and BOYD (1968) have described effects of glutathione on the rate of cholesterol side-chain cleavage by intact mitochondria from the ovaries of intact immature female rats pretreated with the placental gonadotropin, Pregnant Mare Serum Gonadotropin. The results observed may have been due in part to effects on the complex hydroxylation reactions preceding scission of the side-chain and in part on the associated dehydrogenase reaction converting pregnenolone to progesterone (pregn-4-ene-3:20-dione). It was decided to investigate the effects of glutathione on adrenocortical steroid hydroxylations by examining the effects on 11β-hydroxyl-

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ation of 11-deoxycorticosterone(DOC).The results of this investigation would not be influenced by an effect of thiols on the subsequent dehydrogenation reaction as is possible with cholesterol side-chain cleavage.

Materials and Methods

The materials and methods employed in this study have been described previously (WICKRAMASINGHE, 1972).Intact fresh bovine adrenal cortex mitochondria (final concentration, 2 mg protein per ml assay medium) which had been washed twice in 0.154 M-KCl were used. The incubation was carried out for 10 min at 37°C and the corticosterone assayed basically by the method of MATTINGLY(1962) and MATTINGLY et al.(1964).The mitochondria were not preincubated before commencing the assay.

Results

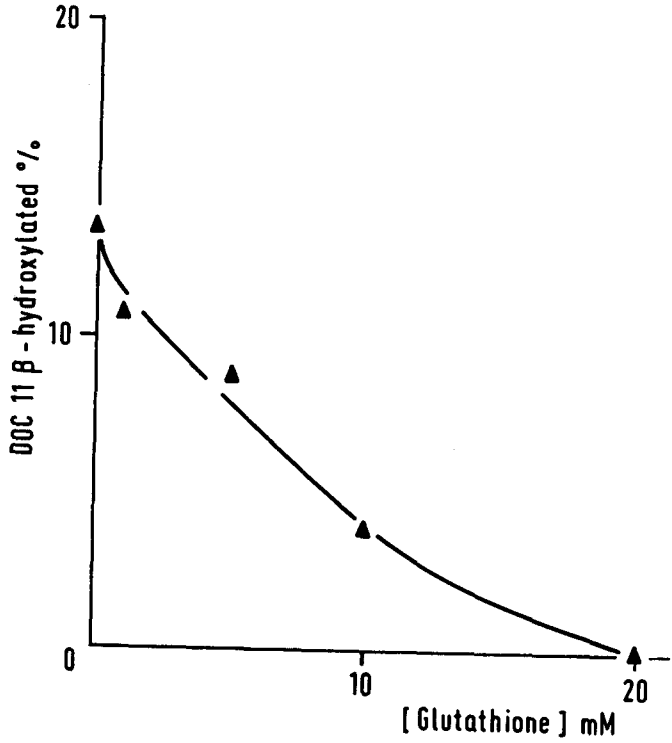

Figure 1. The DOC 11β-hydroxylating activity of intact adrenal cortex mitochondria in the presence of glutathione (GSSG)

It is seen from Fig.1 that the effect of oxidised glutathione (GSSG)on the 11β-hydroxylation of DOC by intact adrenal cortex mitochondria is inhibitory

at all concentrations from 1 to 20 mM.This inhibition is marked at all the concentrations studied and at 20 mM-glutathione steroid hydroxylation activity appears to be arrested.By contrast the inhibitory effect of the higher concentrations (5 - 20 mM) studied of reduced glutathione (GSH) is not as marked and at the lowest concentration (1 mM)used a slight activation of hydroxylation is observed (Fig.2).

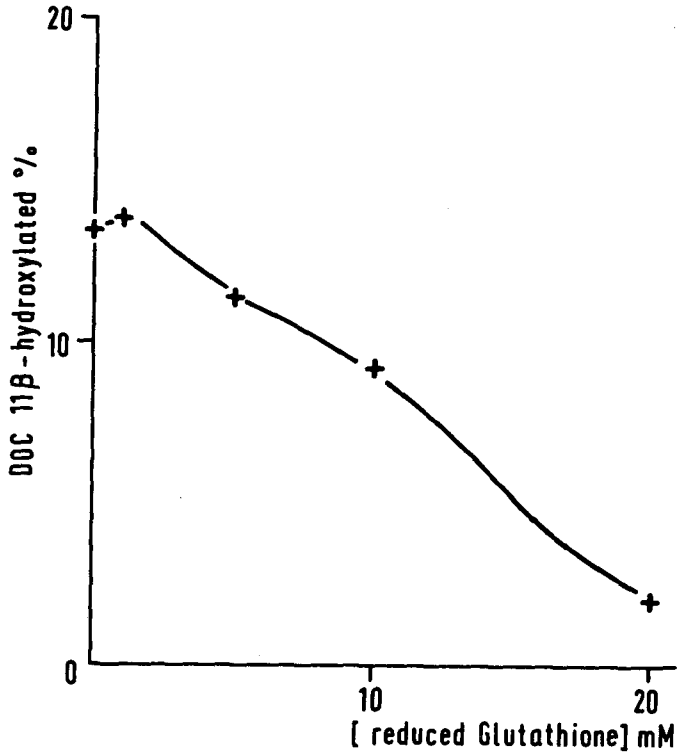

Figure 2. The DOC 11β-hydroxylating activity of intact adrenal cortex mitochondria in the presence of reduced glutathione(GSH)

Discussion

The results obtained and reported here on the effects of the reduced and oxidised forms of glutathione on the 11β-hydroxylation of DOC by intact bovine adrenal cortex mitochondria are similar to those observed on the effects of these thiols on the conversion of cholesterol to pregnenolone and progesterone by intact rat ovarian mitochondria.In the latter case too, low concentrations of GSH appeared to stimulate activity

while higher concentrations of GSH and all concentrations of GSSG were inhibitory.Also both hydroxylations are more effectively inhibited by GSSG than by GSH.

Glutathione is believed to have a biological function in the detoxification of foriegn compounds by the pathway of synthesis of mercapturic acid and possibly in the protection of thiol groups in proteins (BOYLAND and CHASSEAUD,1969;YOUNG and MAW,1958).The slight activation of hydroxylation by low concentrations of GSH may be due to a protection of the labile sulphur groups of adrenodoxin , an iron-sulphur protein component of the mitochondrial steroid hydroxylating enzyme complex (WICKRAMASINGHE,1972), or of the thiol groups of the enzyme components.It may also be due to an effect of the tripeptide on the assay medium (see WICKRAMASINGHE,1972).GSH is also a reducing agent and its thiol group may react(BOYLAND and CHASSEAUD,1969) with the metal (Fe) of the metal-containing hydroxylase components,adrenodoxin and cytochrome P450, thus inhibiting their activity.Finally glutathione is also known to be an effective agent causing mitochondrial swelling which may lead to their lysis(NEUBERT,1966; NEUBERT and LEHNINGER,1962;LEHNINGER and SCHNEIDER, 1959).However, in order to permit comparison with the reported effects of glutathione on cholesterol side-chain cleavage by rat ovarian mitochondria an NADPH-(reduced nicotinamide-adenine dinucleotide phosphate-) generating system was used in the present experiments as sources of reducing equivalents for the hydroxylation reaction.It is believed that intact mitochondrial membranes are impermeable to the reduced form of $NADP^+$. Since in these assays NADPH supported an active rate of steroid hydroxylation without added citric acid cycle intermediates (BROWNIE and GRANT,1954;GRANT and BROWNIE,1955) it may be argued that the mitochondrial membranes were permeable to NADPH and therefore "leaky" or not intact.Furthermore glutathione is known also to have an effect on microsomal hydroxylation reactions (SCHOLAN,1969).

Glutathione is thought to occur in concentrations of 10 - 200 mg per 100 g of tissue (BOYLAND and CHASSEAUD,1969) and the physiological significance under normal conditions of the observations reported here has yet to be assessed.However the present work demonstrates that these thiols have a significant effect on the enzymatic insertion of a hydroxyl function into a steroid by bovine adrenal cortex mitochondria.This could lead to endocrine disorders during elevated levels of detoxification of foriegn compounds by the mercapturic acid pathway.The results also confirm that similar effects described on the side-chain cleavage of cholesterol by intact rat ovarian mitochondria were probably not solely due to an action of the thiols on the conversion of pregnenolone to progesterone

by the 3β-ol dehydrogenase and the Δ^5-3-ketosteroid isomerase but affected the hydroxylation reactions concerned in converting cholesterol to pregnenolone.

Acknowledgements

The author wishes to record his gratitude to Glaxo Laboratories for a Research Fellowship and supporting funds.

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