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The Inhibition by ADP of NADPH-Supported Adrenal Steroid 11β-Hydroxylation[1] (39371)

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Accumulating evidence suggests the possibility that, in the adrenal cortex, the sequence of reactions from cholesterol to corticosterone or cortisol may be functionally a highly integrated system. In addition to the primary control by the adrenocorticotropic hormone (ACTH) which takes place at the transformation of cholesterol to pregnenolone, a series of secondary controls of the steroidogenic pathway appear to be present. Thus, a number of investigations have shown a relationship between steroid 11β-hydroxylation and oxidative phosphorylation (1-5) and steroid 11β-hydroxylation can inhibit the conversion of cholesterol to pregnenolone but the reverse situation does not occur (6). Ca^{2+} has been found to stimulate both pregnenolone synthesis (7) and 11β-hydroxylase (8). Reduced NAD^+ inhibits the formation of progesterone from pregnenolone and this inhibition can be reversed by ascorbic acid and other substances (9). Both NAD^+ and NADH stimulate Δ5-3-keto-steroid isomerase (10).

It has been found recently that the succinate supported 11β-hydroxylation of deoxycorticosterone (DOC) to form corticosterone in rat adrenal mitochondria is inhibited by ADP (11). Much of the data can be explained on the basis of a competition for reducing equivalents between the hydroxylase system and the cytochrome chain. However, some observations suggested the possibility that ADP may also effect the hydroxylase system directly. In this report data is presented which indicate that ADP can indeed directly inhibit steroid 11β-hydroxylation.

Materials and methods. Mitochondria were prepared from rat adrenals as described previously (12). Following the second 0.25 *M* sucrose wash, the mitochondrial pellet was suspended in a volume of 12 m*M* Tris, pH 7.5, equal to that of the original homogenate (90 mg wet weight of tissue per ml) and contained 2.0 to 2.5 mg of protein per ml as determined by the method of Lowry *et al.* (13).

The complete system for the 11-hydroxylation of deoxycorticosterone (DOC) to form corticosterone consisted of 0.2 ml of the mitochondrial preparation, 36 m*M* Tris, pH 7.5, 20 m*M* $CaCl_2$, 30 μ*M* DOC, 0.07 ml of NADPH prepared by a preliminary incubation for 15 min at room temperature of a solution containing, per ml, 25 μmol of $NaNADP^+$, 70 of μmole Na_2 glucose-6-P, and about 2000 units of glucose-6-P dehydrogenase, other additions as indicated and 0.25 *M* sucrose to a final volume of 1.25 ml. The DOC was added in 0.01 ml of ethanol and an incubation control with 0.01 ml of ethanol without DOC was routinely carried out. The incubations were carried out at 20° in a Dubanoff shaking incubator for 6 min, at which time 0.4-ml aliquots were delivered into 3 ml of dichloromethane and, after shaking and centrifugation, 2 ml of the dichloromethane taken for corticosterone determination by a modification (12) of the fluorometric method of Glick *et al.* (14).

Results. The incubation conditions used permit the 11β-hydroxylation of deoxycorticosterone to be supported directly by exogenous NADPH. This would obviate a competition by the hydroxylase and the cytochrome chain for reducing equivalents derived from a Krebs cycle acid such as succinate. It is seen from the data in Table I that 11β-hydroxylase supported by NADPH as the source of electrons is inhibited by ADP and ATP. ADP is more inhibitory than ATP, as was found with intact mitochondria (11) and that a clear inhibition is seen at concentrations as low as 50 μ*M*.

Ca^{2+}, which is present in these incubations, is known to induce swelling in adrenal

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TABLE I. THE EFFECT OF ADP AND ATP ON THE FORMATION OF CORTICOSTERONE FROM DEOXYCORTICOSTERONE.

Nucleotide concentration (m*M*)	ADP		ATP	
	nmole corticosterone per ml	Percentage change	nmole corticosterone per ml	Percentage change
0	13.0		13.0	
0.01	13.0	0	13.4	0
0.05	9.07	−30	12.1	−7
0.10	6.34	−51	11.5	−12
0.50	2.88	−78	7.06	−46
1.0	1.58	−88	4.90	−62

mitochondria (8, 15) and ADP and ATP have been found to inhibit this swelling (15). The inhibition of the 11-hydroxylase by ADP may result from a modification of the mitochondrial membranes so that the entry of NADPH is affected. Several experiments were carried out to explore this possibility. The data in Table II show that the presence of several different types of detergents, which would be expected to disrupt mitochondrial structure, the inhibition by ADP persists. In the presence of some detergents the sensitivity of the hydroxylase to Ca^{2+} changes and Ca^{2+} concentration has been adjusted to obtain maximal activity.

In the experiments presented in Table III the effects on the ADP inhibition of preincubation of the mitochondria under various conditions has been determined. It is seen that the ADP inhibition is still present after a 10-min preincubation in the presence of Ca^{2+}, NADPH, and ADP or in the presence of Ca^{2+} and NADPH. There is a small decrease in the inhibition under the latter preincubation condition.

Discussion. A number of observations indicates that the ADP effect is due to an action directly upon the hydroxylase system. The ADP inhibition persists in the presence of various detergents as well as when the mitochondria are preincubated with Ca^{2+} and NADPH where the possibility of an ADP induced impairment of NADPH entry into the mitochondrial membranes is not present. In support of this conclusion is the observation that while ADP is more inhibitory than ATP, ATP is more effective than ADP in preventing the Ca^{2+} induced swelling of adrenal mitochondria (15). The ADP inhibition of 11β-hydroxylase activity is somewhat less when the mitochondria are preincubated with Ca^{2+} and NADPH (77%) than when ADP is also present during this preincubation (89%). This may indicate a possible minor effect of ADP upon NADPH entry into the mitochondrial membranes. Further work will be required to clarify the mechanism of the ADP inhibition as well as the Ca^{2+} stimulation of this enzyme system.

TABLE II. THE INHIBITION OF 11-HYDROXYLATION BY ADP IN THE PRESENCE OF VARIOUS DETERGENTS.[a]

Addition	ADP		
	nmole corticosterone/ml		Percentage change
Lubrol	18.0	1.8	−90
Triton X-100	13.8	1.5	−89
Deoxycholate	18.1	5.8	−68

[a] ADP, when present, was 2 m*M*. The concentrations of the detergents, per milliliter of incubation medium, were Lubrol, 40 μg; Triton X-100, 80 μg; Na deoxycholate, 80 μM. Ca^{2+} concentrations in incubations with Lubrol was 20 m*M*, and in those with Triton X-100 and deoxycholate were 8 m*M*.

TABLE III. THE EFFECT OF PREINCUBATION IN THE ABSENCE AND PRESENCE OF ADP ON THE INHIBITION OF 11-Hydroxylase by ADP.[a]

Preincubation	ADP		
	nmole corticosterone/ml		Percentage change
A	17.2	1.5	−91
B	22.2	5.2	−77
C	21.3	2.3	−89

[a] Preincubation: A, none. B, 10 min with the complete system except for DOC. ADP, when present, was added in the final incubation. The reaction was started with DOC. C, the preincubation was the same as in B except that ADP, when added, was present during the preincubation. The reaction was started with DOC. ADP, when present, was 2 m*M*.

Summary. The 11β-hydroxylation of deoxycorticosterone to form corticosterone in adrenal mitochondria has been found to be inhibited by ADP and ATP, with ADP being the more inhibitory of the two. The evidence suggests that the ADP directly affects the enzyme system.

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