THE EFFECTS OF STEROID HORMONES ON THE GLUTAMIC DEHYDROGENASE REACTION

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The recent observation that some steroid hormones could act as potent inhibitors of enzymic DPNH oxidation, catalyzed by particulate DPNH-cytochrome C reductase preparations (1), prompted us to investigate the possible metabolic consequences of such an inhibition. The oxidation of glutamate to α-ketogluterate (αKG) and ammonia by DPN (or TPN) was of particular interest because of the importance of glutamic acid in amino acid synthesis and breakdown.

This suggested that in a system which contains both glutamic dehydrogenase (GDH) and DPNH-cytochrome C reductase, the inhibition of the latter reaction by steroids could depress the oxidation of glutamate when DPN was rate-limiting. In accordance with this, 5×10^{-5} M progesterone caused a 2.7-fold increase in the rate of DPNH accumulation in a system consisting of DPN, glutamate, and disrupted liver particles.

When this phenomenon was examined in greater detail, however, it was found that besides their effect on DPNH-cytochrome C reductase, some steroids could inhibit glutamic dehydrogenase directly. This was shown with soluble extracts prepared by freezing and thawing liver mitochondria in distilled water. Any residual DPNH oxidase in these preparations was inhibited by KCN (10^{-3}M). The GDH reaction could then be followed in the spectrophotometer by observing the change in optical density at 340 mμ of the reduced pyridine nucleotides.

Table 1, columns 1 and 2, shows the effect of several steroid hormones on the initial rate of L-glutamate oxidation using DPN as the electron acceptor.

Because of the complicated kinetics of the inhibition, the data (at two steroid concentrations) are presented as per cent inhibition rather than in terms of a constant proportional to the affinity of the enzyme for the steroid. Diethylstilbestrol, estradiol, and progesterone were most inhibitory while the adrenal corticoids were much less effective. Qualitatively similar results were observed when TPN was used instead of DPN. The reverse reaction, reductive amination of αKG, was also inhibited by these hormones.

TABLE 1

EFFECT OF STEROIDS ON GLUTAMATE OXIDATION

	Rat GDH[1]		Beef GDH[2]	
	Steroid Concentration			
	5×10^{-5} M.	1×10^{-6} M.	5×10^{-5} M.	1×10^{-6} M
	% Inhibition			
Diethylstilbestrol	94	87	97	28
Estradiol	95	6	72	0
Progesterone	97	12	58	0
Δ^4 Androstene 3,17 dione	69	8	66	0
Testosterone	-	-	44	0
DOC	62	0	8	0
Corticosterone	37	12	0	0
Cortisol	50	0	0	0

[1] Reaction mixture contained soluble mitochondrial extract of rat liver equivalent to 2.5 mg of tissue, 0.1 ml 50% propylene glycol with or without added steroid; L-glutamate 10×10^{-2} M; DPN 2×10^{-4} M, KCl 6×10^{-3} M, KCN 10^{-3} M, phosphate buffer 2×10^{-3} M, pH 7.5 in a total vol. of 2.5 ml. Reaction run at room temperature and the initial rate estimated from the ΔOD at 340 mμ in the 15-30 sec. interval.

[2] Reaction mixture contained 10 μg beef liver GDH, 0.1 ml 50% propylene glycol with or without steroid, L-glutamate 5×10^{-2} M, DPN 2×10^{-4} M, Tris buffer 10^{-2} M pH 8.0, and EDTA 10^{-4} M in 2.5 ml volume. Initial rate determined as above.

The crystalline glutamic dehydrogenase of beef liver (obtained from the Sigma Chemical Company as a suspension in sodium sulfate) was then examined, Table 1, columns 3 and 4. As with the rat liver enzyme several of the steroids were potent inhibitors of glutamate oxidation. In this connection, it is of interest that Kielley (2) observed that carcinogenic fluorene derivatives inhibited crude rat liver glutamic dehydrogenase but did not affect either a more purified preparation from the same species or the crystalline enzyme from beef liver.

Glutamic dehydrogenase activity in crude extracts of S. typhimurium, measured by TPN reduction, was not affected by 1×10^{-5} M diethylstilbestrol.

Kinetic studies of both the forward and reverse reactions, catalyzed by the crystalline enzyme, showed that the steroid inhibition was not clearly competitive with either the substrates or the pyridine nucleotides. At a given steroid concentration, the degree of inhibition of glutamate oxidation increased greatly

TABLE 2

EFFECT OF ADP ON STEROID INHIBITION OF GDH[1]

	ΔOD/min 340 mμ x 10^3			
	TPN		TPNH	
ADP	Control	Estradiol 2×10^{-5} M	Control	Estradiol 2×10^{-5} M
0	128	88	76	28
1×10^{-5}	166	156	128	64
5×10^{-5}	-	-	200	140
1×10^{-4}	-	-	276	256

[1] Reaction run as in 2, Table 1 with TPN or TPNH in lieu of DPN.

over the temperature range from 6 to 30° C. Steroid inhibition was also enhanced by raising the pH from 7 to 8.6.

On the basis of Frieden's report (3) that adenine nucleotides stimulated GDH, we investigated the influence of these compounds on the steroid-inhibited reaction. ADP completely reversed the steroid effect on both glutamate oxidation and αKG reduction, although ten times as much ADP was required to reverse estradiol inhibition of the latter reaction. Table 2 illustrates these findings using TPN and TPNH. Similar results were obtained with DPN and DPNH.

Engel, Scott and Colman (4) have recently reported a 10-15% stimulation of the GDH reaction in the presence of very low concentrations of corticosterone or cortisol. Under our conditions, no consistent stimulation was observed with the crystalline enzyme.

The finding that some steroids can directly affect the GDH reaction provides a means by which these hormones may possibly regulate intermediary metabolism. The additional observation that ADP can reverse the steroid inhibition suggests that the intramitochondrial concentration of this nucleotide might play a part in controlling the magnitude of the steroid effect. Since ADP was considerably less effective in reversing the inhibition of αKG reduction than of glutamate oxidation, the interaction of steroid and nucleotide could, perhaps, influence the direction of carbon flow through the GDH reaction.

REFERENCES

1. Yielding, K. L., and Tomkins, G. M., Proc. Nat. Acad. Sci., 45, 1730 (1959).

2. Kielley, R. K., J. Biol. Chem., 227, 91 (1957).

3. Frieden, C., J. Biol. Chem., 234, 815 (1959).

4. Engel, L. L., Scott, J. F., and Colman, R. F., Fed. Proc., 19, 159 (1960).