ACTH ACTIVATION OF GLYCOLYSIS IN THE RAT ADRENAL GLAND*

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SUMMARY

A number of substrates of glycolysis, the pentose phosphate pathway, the Krebs cycle and the pyridine and adenine nucleotides (including cyclic AMP) have been measured before and after ACTH stimulation in hypophysectomized rats. Crossovers in substrate concentrations at the level of phosphofructokinase and glyceraldehyde phosphate dehydrogenase were found, indicating an activation of these enzymes in the stimulated adrenals. Elevations in cyclic AMP and a decrease in the ATP/ADP·AMP ratio were also found, either of which could explain phosphofructokinase activation.

Control of steroidogenesis by regulation of NADPH generation has been suggested by the studies of Haynes et al., who showed an increase in 3'5'AMP and an activation of phosphorylase (1), McKerns, who showed an activation of glucose-6-phosphate dehydrogenase (2), and Ferguson, who showed a bypass of puromycin inhibition of steroidogenesis by NADPH generation systems (3). We have considered two possibilities for the interaction of energy metabolism with steroidogenesis: first, that glycolysis and oxidative metabolism might be activated by the energy demands of hydroxylation through adenine nucleotide and citrate control of phosphofructokinase similar to that postulated for other tissues (4); and second, that direct activation of enzymes concerned with substrate

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flow to appropriate dehydrogenases might initiate hydroxylation. In the following studies, evidence for perturbations in energy metabolism was obtained by measuring substrates of glycolysis, the pentose phosphate pathway, the Krebs cycle, and pyridine and adenine nucleotides (including cyclic AMP), before and after ACTH stimulation.

MATERIALS AND METHODS

Left adrenal glands from four-hour hypophysectomized female rats were collected into liquid nitrogen following 100 mμ ACTH I.V. Right adrenal glands removed prior to this were used as controls.

Neutralized 5% perchloric acid extracts were assayed fluorometrically for the various intermediates using specific enzymes coupled to pyridine nucleotide oxidation or reduction. Cyclic AMP was measured using the enzymatic radioisotopic displacement method (5).

RESULTS AND DISCUSSION

Figure 1 shows a summary of the changes in glycolytic intermediates after ACTH stimulation. A decrease in the hexose phosphates, G1P,* G6P and F6P, with a simultaneous increase in FDP and DAP beginning at 20 seconds and becoming maximal at two minutes, is clearly shown. This crossover in substrate concentrations between F6P and FDP suggests an activation of phosphofructokinase.

After two minutes' stimulation, the concentration of GAP

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*G1P, glucose-1-P. Other abbreviations: G6P, glucose-6-P; F6P, fructose-6-P; FDP, fructose-1,6-di-P; GAP, glyceraldehyde-P; 3PGA, 3-P-glyceric acid; PEP, phosphoenolpyruvate; DAP, dihydroxyacetone-P; GAPDH, glyceraldehyde-P dehydrogenase; PFK, phosphofructokinase; Pyr., pyruvate.

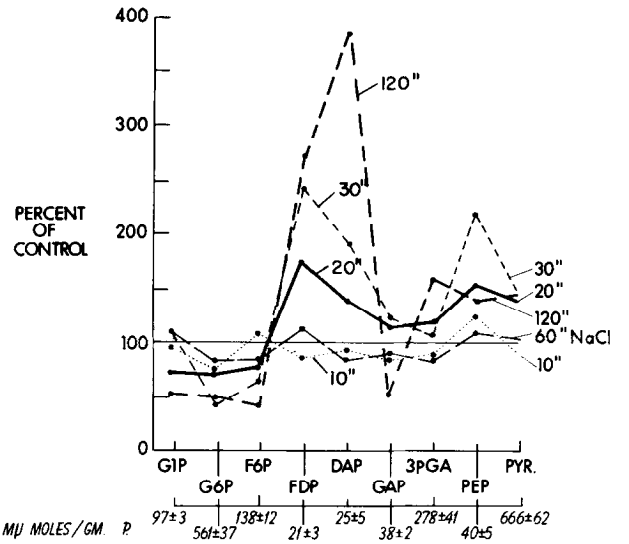

Figure 1. The Effect of 100 mμ ACTH on Glycolytic Intermediates at Various Time Intervals. Values of the glycolytic intermediates are expressed as a percentage of the control values obtained before ACTH infusion. The concentration of each intermediate in mμmoles per gram protein with its standard error of the mean is shown for the control glands along the bottom of the figure. The control and stimulated values for each experiment were obtained from pooled adrenals from at least 20 rats.

returns to the control level in the stimulated glands. This is followed by a significant increase in 3PGA, PEP and pyruvate. The relative fall in GAP in relation to DAP, along with the increase in the remaining three carbon acids of the glycolytic system, is another crossover in substrate concentrations and indicates an activation of glyceraldehyde phosphate dehydrogenase.

In other tissues, phosphofructokinase is known to be a rate-limiting enzyme for glycolysis and to be under allosteric control by a number of effectors including AMP, inorganic phosphate, citrate and, in some instances, cyclic AMP (4). Unlike PFK, the maximal catalytic activity of GAPDH is high in most tissues and

Table I

EFFECTS OF ACTH ON LEVELS OF CITRIC ACID CYCLE INTERMEDIATES 6-P-GLUCONATE, PYRIDINE AND ADENINE NUCLEOTIDES

Rat Adrenal Component	Control	ACTH, 2 minutes
	mμmoles/gm Protein	
Citrate	2,472 ± 456	2,114 ± 468
Isocitrate	298 ± 41	303 ± 36
α-Ketoglutarate	149 ± 13	160 ± 14
Malate	1,226 ± 147	1,092 ± 187
Oxaloacetate	31 ± 3	40 ± 5
6-P-Gluconate	55 ± 9	57 ± 8
NAD	2,400 ± 178	2,625 ± 354
NADH	237 ± 40	238 ± 22
NADP	397 ± 53	453 ± 58
NADPH	403 ± 50	468 ± 50
ATP	15,483 ± 513	11,833 ± 379
ADP	1,760 ± 215	1,438 ± 231
AMP	521 ± 120	947 ± 320

Table I. Values shown are means ± standard error of the mean.

on this basis it would not appear to be a likely regulatory site in glycolysis. A regulatory role has been observed, however, in aerobic-anaerobic transitions in brain and heart tissues (6). Presumably the NADH:NAD ratio mediates this control by regulating the rate of this oxidative reaction.

Similar studies of the Krebs cycle intermediates--citrate, isocitrate, α-ketoglutarate, malate and oxalacetate, the pyridine and adenine nucleotides, and the first member of the pentose phosphate cycle, 6-phosphogluconate--are shown in Table 1. No significant changes in the Krebs cycle or pyridine nucleotides were detected at two minutes after ACTH stimulation. Since it is highly likely that an increase in rate of some of the enzymes associated with these intermediates and cofactors occurs, we suggest that none of the enzymes of the Krebs cycle or malic enzyme are rate-limiting. In addition, changes in the pyridine nucleotide redox state could have been masked by reciprocal changes occurring in the cytoplasm and mitochondria. A decrease in the ATP/ADP·AMP ratio after ACTH stimulation was found and could account for PFK activation at least in the two-minute stimulated adrenals.

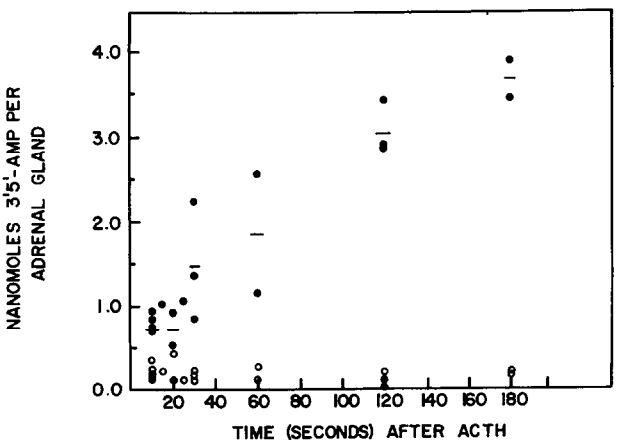

Figure 2. The Effect of 100 mμ ACTH on Cyclic AMP Levels. Each point represents the assay of one adrenal gland (approximately 25 mg) indicated in nanomoles on the ordinate. The open circles show the control levels, and the filled circles, the stimulated levels. The horizontal bars at each time period represent the means of the stimulated values.

The cyclic AMP response to ACTH infusion is shown in Figure 2. A significant and almost linear increase in cyclic AMP is shown beginning at ten seconds after ACTH infusion (the earliest time period examined) and continues through the three-minute period. Grahame-Smith et al. have observed an increase in cyclic AMP one minute after ACTH administration (7). The time relationship of this change to PFK activation is compatible with cyclic AMP functioning as a mediator of this phenomenon. However, whether or not an activation of glycolysis mediated by cyclic AMP increases steroid hydroxylation by providing reducing equivalents, or whether a stimulation of the steroid hydroxylating enzymes per se mediates this activation of glycolysis via a decrease in the ATP/ADP·AMP ratio, has not been settled.

REFERENCES

1. Haynes, R. C., Jr., J. Biol. Chem., 233, 1220 (1958).
2. McKerns, K. W., Biochim. Biophys. Acta, 90, 357 (1964).
3. Ferguson, J. J., Jr., Biochim. Biophys. Acta, 57, 616 (1962).
4. Passonneau, J. V. and Lowry, O. H., in "Advances in Enzyme Regulation," edited by G. Weber, vol. 2, Macmillan Company, New York, 1964, p. 265.
5. Brooker, G., Thomas, L. J., Jr. and Appleman, M. M., Biochemistry, 7, 4177 (1968).
6. Lowry, O. H., Passonneau, J. V., Hasselberger, F. X. and Shulz, D. W., J. Biol. Chem., 239, 18 (1964).
7. Grahame-Smith, D. G., Butcher, R. W., Ney, R. L. and Sutherland, E. W., J. Biol. Chem., 242, 5535 (1967).