SOME EFFECTS OF GLUCOCORTICOIDS ON THE SUBCELLULAR DISTRIBUTION OF THE ACTIVITIES OF CITRATE SYNTHASE AND PHOSPHOENOLPYRUVATE CARBOXYKINASE IN LIVERS OF RATS AND COWS

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1. Introduction

Oxaloacetate is converted to phosphoenolpyruvate or condenses with acetyl-CoAS to form citrate in the hepatic cell.

In rat liver the key gluconeogenic enzyme, phosphoenolpyruvate carboxykinase (PEPCK) (EC 4.1.1.32), is mostly in the cytosol [1], while citrate synthase (CS) (EC 4.1.3.7) is located in the mitochondrion. However, in bovine liver, these two enzymes are more equally distributed between the compartments [2].

In rat liver the administration of a glucocorticoid [3, 4] resulted in an increase in the activity of the enzyme PEPCK which can be correlated with the amount of glucose synthesised. In the liver of the cow a striking difference was observed where the effect of glucocorticoids, ketosis and starvation appeared to be accompanied by a depression of PEPCK activity [5, 6].

This paper examines the hypothesis that the increase in gluconeogenesis in rats, following glucocorticoid administration, is related to an increase in cytoplasmic PEPCK activity and a depression of CS activity, whereas the antiketogenic action of a glucocorticoid in bovine liver is accompanied by a depression of cytoplasmic PEPCK activity and an increase in CS activity. The effect of glucocorticoids on glutamate dehydrogenase (EC 1.4.1.2) is also described in both rat and bovine liver.

2. Materials and methods

2.1. *Animals*

Female rats (105–185 g) were fed *ad lib.* with a standard diet until sacrificed. 1 mg dexamethasone-21-isonicotinate or 1 mg betamethasone suspended in 1 ml of physiological saline was injected intramuscularly. The animals were sacrificed 24 hr and 48 hr later. The liver was removed and placed in cold 0.32 M sucrose.

Cows which had had at least 2 calves and were in the first 3 months of lactation were housed and fed normal rations as described previously [5]. The animals were injected intramuscularly with dexamethasone-21-isonicotinate (10 mg) and liver tissue was surgically removed [5] 48 hr after injection. The tissue was placed in cold 0.32 M sucrose for subsequent assay of enzyme activity.

2.2. *Preparation of particulate and soluble fractions*

10% (w/v) homogenates of liver in 0.32 M sucrose were obtained by subjecting the tissue to 10 up and down strokes at low revolutions in a Potter-Elvehjem homogeniser with teflon pestle. They were centrifuged at 100,000 *g* (50 rotor in Beckmann Model L ultracentrifuge) at 2° for 30 min.

The pellets were resuspended in the initial volume of 0.32 M sucrose and were sonicated at 8.4 A (Dawe Soniprobe, Dawe Instruments Ltd. London) for 12 × 15 sec periods with intervals of 15 sec for cooling. The sonicated material was centrifuged at 85,000 *g* (50 rotor Beckmann Model L ultracentrifuge) at 2° for 20 min. The supernatants obtained were used for assay of the soluble and particulate fractions, respectively.

The activity of CS was assayed essentially by the method of Srere et al. [7] and PEPCK and glutamate dehydrogenase by reported methods [5, 8].

Probability values of P were obtained using Students t test.

3. Results

The changes in the activities of the enzymes, PEPCK, CS and glutamate dehydrogenase in the livers of rats and cows after glucocorticoid administration are shown in table 1.

The effects on PEPCK activity in rat liver of a dexamethasone ester or betamethasone were identical at 24 hr and 48 hr after injection. The activity of the cytoplasmic enzyme increased to approximately 155% and 300% of control value, 24 hr and 48 hr respectively, after steroid injection. This presumably ruled out any possible side effect of the isonicotinate group of the dexamethasone ester.

PEPCK was evenly distributed between the particulate (53%) and cytoplasmic (47%) fractions in bovine liver. In bovine liver, the PEPCK activity of the particulate fraction was not changed by glucocorticoid administration, however the cytoplasmic enzyme activity was depressed to 36% of control value.

The administration of either dexamethasone-21-isonicotinate or betamethasone depressed the activity of particulate CS in rat liver by one third after 24 hr and 48 hr. CS activity was almost exclusively in the particulate fraction of rat liver and was distributed in the same way as the glutamate dehydrogenase. However in the cow, if the values of the two enzymes CS and glutamate dehydrogenase represent true *in vivo* activities and both are assumed to leak from the mitochondria quantitatively to the same degree, then CS is probably present in the cytoplasm of the intact cell in bovine liver. 48 hr after the administration of dexamethasone-ester the activity of CS in bovine liver increased by 78% in the particulate fraction and 87% in the cytoplasmic fraction.

Table 1
Activities of enzymes in cell fractions of livers of cows and rats before and after administration of a glucocorticoid.

	Hormone	Control		24 hr after glucocorticoid administration		48 hr after glucocorticoid administration	
		P	C	P	C	P	C
		μmole × g^{-1} × min^{-1}					
Phosphoenolpyruvate carboxylase							
Cow	DE	7.14	6.22	–	–	7.95	2.22***
Rat	DE	0.53	1.39	0.43 (2)	2.14 (2)	0.44 (2)	4.22 (2)
Rat	B	–	–	0.51	2.18**	0.42 (2)	3.92 (2)
Citrate synthase							
Cow	DE	2.12	0.38	–	–	3.78**	0.71*
Rat	DE	15.1	0.37	11.3***	0.55	11.7***	0.41
Rat	B	–	–	11.2***	0.41	12.4***	0.31
Glutamate dehydrogenase							
Cow	DE	29.2	2.77	–	–	49.6***	2.65
Rat	DE	71.9	1.07	72.9	1.31	65.3*	0.62
Rat	B	–	–	61.5***	1.34	62.4***	0.96

Activities are expressed as μmole substrate consumed or product formed at 37°. The values are the means of at least 4 animals unless indicated by values in parentheses. DE is dexamethasone-21-isonicotinate, dose: 10 mg/cow, and 1 mg/rat; B is betamethasone, dose: 1 mg/rat. The livers were obtained from the cows by biopsy and from killed rats by excision. P is particulate fraction and C the cytoplasmic fraction.

***$P < 0.001$, **$P < 0.01$, *$P < 0.05$ compared with control values.

Glutamate dehydrogenase activity almost doubled in bovine liver following glucocorticoid administration whereas only small changes in activity were observed in the rat liver.

4. Discussion

The effects of a glucocorticoid on enzyme activities were markedly different in rat liver compared with bovine liver. Adult bovine liver is continually in the gluconeogenic state while rat liver oscillates between the conditions of gluconeogenesis and glycolysis, being dependent on the physiological and nutritional state of the animal.

Several reports (for review see [4]) have shown that gluconeogenesis is increased in the rat following glucocorticoid administration. It is believed that this increase can be related to the increased activity of a key gluconeogenic enzyme, PEPCK. Our results confirm these findings. Furthermore, the activity of citrate synthase was depressed in the mitochondrial fraction of rat liver 24 hr after glucocorticoid administration and had not returned to the control value after a further 24 hr period. This depression of CS activity would favour the flow of oxaloacetate to phosphoenolpyruvate.

If there is an increase in gluconeogenesis following glucocorticoid administration in the rat, then in order for there to be an opposite effect in the cow one might expect the activities of PEPCK and CS to change in the opposite direction following steroid treatment. The results clearly show that PEPCK was significantly decreased in the cytoplasmic fraction, while citrate synthase was significantly increased in both particulate and cytoplasmic fractions. Glucocorticoids have an antiketogenic role in the cow [9] and these changes in enzyme activity would support this theory. Furthermore, a recent study [10] has shown that the formation of glucose from the level of malate occurs predominantly in the cytosol fraction of sheep liver. If the same is true for bovine liver, then the cytoplasmic PEPCK is a key gluconeogenic enzyme. It is very interesting that glucocorticoids depress the activity of cytoplasmic PEPCK and not the activity of mitochondrial PEPCK. Although the evidence presented here and elsewhere [5, 9] is circumstantial, because no rate measurements are available, gluconeogenesis may be decreased in bovine liver following glucocorticoid administration.

The depression of PEPCK in the cytoplasmic enzyme and not in the particulate enzyme in the bovine liver following glucocorticoid administration might be explained on the basis that they are different enzymes as observed in sheep liver [11].

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