Vol. 115, No. 2, 1983 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
September 15, 1983 Pages 606-610

DIRECT INFLUENCE OF DEXAMETHASONE IN VIVO ON RAT TESTICULAR LIPOGENIC ENZYMES INVOLVED IN PYRUVATE-MALATE PATHWAY

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Received August 5, 1983

SUMMARY: The changes in specific activities of NADP-isocitrate dehydrogenase, ATP-citrate lyase, Malate dehydrogenase and malic enzyme were studied in adrenalectomised and dexamethasone injected pubertal rats. One month after adrenalectomy the specific activity of NADP-isocitrate dehydrogenase increased but the specific activities of the other three enzymes decreased. An opposite effect was seen after dexamethasone administration to intact animals. The changes observed in the specific activities of enzymes of adrenalectomised and dexamethasone treated animals reverted back to normal after dexamethasone replacement and withdrawal, respectively.

INTRODUCTION: Lipids and carbohydrates are the two major energy sources in the testis and are biochemically closely interlinked (1). A group of functionally related enzymes involved in pyruvate/malate cycle is concerned with lipogenesis in testis, adrenal, ovary and other tissues (2). Isocitrate dehydrogenase ($NADP^+$) providing NADPH and carbon building blocks necessary for lipogenesis has been reported in rat testis (3). The cytoplasmic ATP-citrate lyase in rat testis is thought to be involved in the generation of acetyl-CoA and oxaloacetate for lipogenesis (1,2). Malic enzyme is a potent generator of NADPH necessary for lipogenesis and its activity is more than double that of the pentose phosphate pathway in rat testis (4). Malate dehydrogenase which is involved in the utilisation of oxaloacetate and production of malate for the action of malic enzyme has also been reported in rat testis (4,5). Even though these enzymes are functionally related, their responses to gonadotropins and androgens differ (2,6). Since very little information is available on the influence of adrenal cortical hormones on these enzymes of the testis, we

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0006-291X/83 $1.50

studied the effect of adrenalectomy and dexamethasone administration on specific activities of these enzymes in testis of pubertal rats.

MATERIALS AND METHODS: Male albino pubertal rats (60 days old) of Wistar strain were used in the present investigations. They were housed in a well-ventilated, temperature controlled room with 14 h light and 10 h dark controlled light schedule fed with pelleted diet (Hindusthan Lever) and given tap water ad libitum. The adrenalectomised rats alone were given 0.9% sodium chloride (w/v) as drinking water.

Thirty animals were subjected to total bilateral adrenalectomy (Adx) under ether anesthesia and another thirty were sham-operated (Shm) to serve as controls. 15 adrenalectomised and 15 control rats were killed after 30 days. Rest of the adrenalectomised animals were given dexamethasone (Merck Sharpe and Dohme, India) 5 µg/100 g body weight per day, im, starting on the 31st day post-surgery, for 30 days and the sham operated controls were given physiological saline (0.89% NaCl W/V, im). All animals were killed 24 h after the last injection. (Varying doses of dexamethasone 1 µg, 25 µg, 5 µg, 7 µg and 10 µg per 100 g body weight were tried and 5 µg/100 g body weight was chosen as optimal after assessing the plasma ACTH level).

The completeness of adrenalectomy and the presence of any ectopic adrenal gland were confirmed by measuring the serum concentrations of corticosterone and plasma ACTH, ten days after operation. The animals showing normal or near normal levels of corticosterone and low level of ACTH were discarded and replaced by new batches of animals.

Another group of 30 intact animals were given dexamethasone (25 µg/100 g body weight, im) for 30 days. Same number of controls were injected the vehicle (0.89% NaCl W/V, im) only. 15 controls and 15 experimental animals were killed on the 31st day. The rest were allowed to live further for a period of 30 days without any injection and killed. As soon as the animals were sacrificed, the testes were removed and processed for biochemical investigations.

To determine ATP-citrate lyase (EC 4.1.3.8) activity, the testicular tissue was homogenised with 0.4 M KCl in 20% ethanol. The KCl-ethanol temperature was 0°C but homogenisation was allowed to proceed for 2 min at room temperature. Subsequent operations were carried out at 0°C. After homogenisation, extract was allowed to stand for 5 min, the fatty layer that had risen to the top was skimmed off and the homogenate was centrifuged at 100,500 x g for 15 min. The supernatant was poured through several layers of cheese cloth and the filtrate was used as enzyme extract. The enzyme activity was measured following the method of Srere (7).

NADP-isocitrate dehydrogenase (EC 1.1.1.42) (NADP-ICDH) malate dehydrogenase (EC 1.1.1.37) (MDH) and malic enzyme (EC 1.1.1.40) were extracted in 0.1 M triethanolamine buffer, pH 7.5. The tissue was homogenised in the buffer, homogenate centrifuged at 100,500 x g for 15 min at 4°C and the supernatant was used for enzyme assays. NADP-isocitrate dehydrogenase was assayed according to the method of Bernt and Bergmeyer (8). For determination of malate dehydrogenase and malic enzyme activities, the methods described by Ochoa (9,10) were used.

All enzyme activities were expressed as unit (U) per mg protein. One enzyme U is defined as the amount of enzyme required to convert 1 µM of substrate to product under the described conditions. Protein was measured by the method of Lowry et al. (11).

All substrates, enzymes and other chemicals used were supplied by Sigma Chemical Company (St. Louis, USA). The results were statistically analysed using students 't' test.

RESULTS: One month after adrenalectomy the specific activity of testicular NADP-ICDH increased ($p < 0.05$) but of ATP-citrate lyase ($p < 0.05$), MDH ($p < 0.05$) and malic enzyme ($p < 0.05$) decreased. The specific activities of all these enzymes returned to normal one month after dexamethasone replacement (Table 1). Administration of dexamethasone to intact rats decreased the specific activities of testicular NADP-ICDH ($p < 0.01$) while the specific activities of ATP-citrate lyase ($p < 0.01$), MDH ($p < 0.01$) and malic enzyme ($p < 0.05$) increased. One month after withdrawal of dexamethasone injection the specific activities of all these enzymes reverted back to normal levels (Table 1).

DISCUSSION: The data suggests a differential response by enzymes of the pyruvate-malate pathway to adrenalectomy and dexamethasone treatment. However, such a differential response may lead to a beneficial effect on lipogenesis in adrenalectomised animals and to a deleterious effect in dexamethasone treated intact animals. Isocitrate dehydrogenase is one of the major controlling factors of lipogenesis as it supplies the NADPH and the precursors of carbon building blocks for lipid synthesis. Since the activity of this enzyme increases and decreases after adrenalectomy and dexamethasone administration to intact animals respectively, a corresponding change may be expected in the lipid synthesis. The other three enzymes studied responded together as a single unit.

As mentioned earlier malic enzyme is also a potent generator of NADPH needed for lipogenesis but the decrease in activity observed after adrenalectomy may be compensated by the increased activities of pentose-phosphate pathway enzymes (12) which also generate NADPH.

The data also show that dexamethasone and adrenal hormones decrease the specific activity of testicular NADP-ICDH and increase the specific activities of ATP-citrate lyase, MDH and malic enzyme as the activities of these enzymes are altered in the presence or absence of these steroids. This may be a direct action of glucocorticoids or dexamethasone. Earlier in vitro and in vivo studies have shown a direct inhibitory effect of dexamethasone on the rate of

TABLE 1. Effect of Adrenalectomy, Dexamethasone replacement (5 mg/100 g body weight), Dexamethasone treatment to intact rats (25 mg/100 g body weight) and Dexamethasone withdrawal on the specific activities (U/mg protein) of pubertal rat testicular enzymes.

Group	Status of animals	NADP-ICDH	ATP-citrate lyase	Malate dehydrogenase	Malic enzyme
Sham	Control	0.041 ± 0.008	0.015 ± 0.004	0.325 ± 0.013	0.035 ± 0.005
I					
Adrenalectomy	Experimental	0.062 ± 0.004*	0.003 ± 0.002*	0.289 ± 0.009*	0.019 ± 0.004*
Sham + NaCl	Control	0.040 ± 0.008	0.026 ± 0.006	0.286 ± 0.011	0.039 ± 0.008
II					
Adrenalectomy + Dexamethasone	Experimental	0.043 ± 0.010	0.023 ± 0.007	0.273 ± 0.018	0.033 ± 0.007
NaCl	Control	0.045 ± 0.004	0.013 ± 0.003	0.340 ± 0.010	0.035 ± 0.007
III					
Dexamethasone (Dex)	Experimental	0.021 ± 0.006**	0.024 ± 0.002**	0.387 ± 0.012**	0.065 ± 0.008*
NaCl Withdrawal	Control	0.038 ± 0.008	0.015 ± 0.006	0.288 ± 0.020	0.036 ± 0.009
IV					
Dex. withdrawal	Experimental	0.027 ± 0.010	0.021 ± 0.007	0.271 ± 0.014	0.029 ± 0.017

Each value is mean ± S.E.M. of 15 animals. *$p < 0.05$ **$p < 0.01$

DNA, protein and steroidogenesis and hCG binding capacity to LH receptors (14,15). However, the mechanism of action of the adrenal glucocorticoids and dexamethasone in altering the testicular enzyme activities observed in the present study is not clear.

ACKNOWLEDGEMENT: The help of Mrs. Karen Dove in typing this script is gratefully acknowledged.

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