DEPENDENCE ON INSULIN OF THE HYDROCORTISONE EFFECT ON MUSCLE GLYCOGEN CONTENT

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Received 11 June 1969

1. Introduction

Adrenalectomy does not modify the glycogen content of skeletal muscle in fed salt-treated animals [1] while glucocorticoid administration causes a marked increase in glycogen content of various rat muscles [2–4] evident after 12 h [2]. Both these features suggest that the effect of glucocorticoids on muscle glycogen is indirect, as it has been recently observed for the effects of these hormones on liver glycogen synthesis [5].

In this paper we show that the effect of hydrocortisone on muscle glycogen is abolished in the alloxan diabetic animal. Furthermore in normal animals administration of this cortical hormone causes an early increase in insulinemia. These data indicate that this effect of hydrocortisone is mediated by insulin.

2. Materials and methods

Male Wistar rats (Morini, S.Polo d'Enza) weighing about 250 g were used. Adrenalectomy was performed by the dorsal approach 2 or 4 days before experiments. Diabetes was obtained by alloxan intravenous injection (40 mg/kg). Only glycosuric (more than 5 g of glucose excreted/day) and markedly hyperglycemic animals were used.

Hydrocortisone (5 mg/rat) suspended in physiological saline was injected subcutaneously.

Rats were anaesthetized with Nembutal (5 mg/100 g b.w.), the muscles quickly removed, weighed on a torsion balance and put in boiling 30% KOH. After 30 min hydrolysis glycogen was purified by two precipitations with 1.25 vol of 95% ethanol and colorimetrically determined by the anthrone method [6].

Glucose levels were determined on heart blood samples by glucose-oxidase [7]; on serum from the same samples insulin was determined according to the radioimmunological method of Hales and Randle [8].

3. Chemicals

Hydrocortisone, glucose-oxidase, peroxidase, dianisidine were purchased from Sigma Chem. Co. (St. Louis, Mo.), alloxan from Merk (Darmstadt). For determination of blood insulin levels the Kit CEA-CNEN-SORIN (Sorin, Saluggia) was used. All chemicals used were of analytical grade.

4. Results

Content of glycogen is not modified by adrenalectomy (table 1) whereas it is significantly increased in all muscles sampled after hydrocortisone treatment. However the response to the hormone is significantly different in the various muscles examined: in levator ani muscle the peak of muscle glycogen concentration is reached after 12 h, and subsequently the levels decline, whereas in the other muscles the maximum values are kept at least until 24 h. This difference can be related to the greater glycolytic rate of a totally white muscle, like the levator ani [9].

Table 2 shows that experimental diabetes does not modify substantially muscle glycogen content one week after alloxan injection. However muscle glycogen

Table 1
Effect of hydrocortisone (5 mg/rat) on glycogen content in various rat muscles (mg glycogen/g muscle wet weight). Means ± S.E.M. In parentheses the number of experiments.

Treatment	Hydrocortisone	Levator ani	Soleus	Obliquus externus	Diaphragm
Intact	0	4.67 ± 0.29(5)	5.10 ± 0.69(5)	3.88 ± 0.42(5)	2.44 ± 0.30(5)
Adrenalectomized	0	5.01 ± 0.36(6)	4.27 ± 0.76(6)	5.31 ± 0.77(6)	3.72 ± 0.86(6)
Intact	12 h	6.89 ± 0.33(6)	7.52 ± 1.00(6)	8.59 ± 0.64(6)	6.78 ± 0.69(6)
Adrenalectomized	12 h	10.2 ± 0.85(6)	10.01 ± 1.39(6)	8.05 ± 0.95(6)	8.93 ± 0.98(6)
Intact	24 h	5.72 ± 0.59(6)	10.41 ± 1.70(6)	10.58 ± 2.73(6)	8.71 ± 2.50(6)
Adrenalectomized	24 h	7.38 ± 0.64(5)	6.94 ± 0.59(5)	11.00 ± 1.62(5)	7.71 ± 1.03(5)
Statistical analysis (F test)					
Effect of adrenalectomy		$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$
hydrocortisone		$P \ll 0.01$	$P \ll 0.01$	$P < 0.01$	$P < 0.01$
Interactions hydrocort. X adr.		$P < 0.01$	$P > 0.05$	$P > 0.05$	$P > 0.05$
hydrocort. X time		$P < 0.05$	$P < 0.05$	$P > 0.05$	$P > 0.05$
hydrocort. X adr. X time		$P < 0.05$	$P < 0.05$	$P > 0.05$	$P > 0.05$

Table 2
Effect of hydrocortisone (5 mg/rat 12 h before sacrifice) on glycogen content of muscles of diabetic animals (mg glycogen/g muscle wet weight). Means ± S.E.M.; in parentheses the number of experiments.

Treatment	Levator ani	Soleus	Obliquus externus	Diaphragm
Intact	5.41 ± 0.34(6)	6.73 ± 0.44(6)	7.97 ± 0.76(6)	6.65 ± 0.50(6)
Intact-hydrocortisone	8.00 ± 0.38(6)	10.3 ± 2.11(6)	12.0 ± 0.90(6)	12.0 ± 1.65(6)
Diabetes	5.48 ± 0.53(8)	6.28 ± 0.84(8)	9.01 ± 0.65(8)	3.85 ± 0.51(8)
Diabetes-hydrocortisone	5.70 ± 0.98(6)	5.60 ± 0.31(7)	10.2 ± 0.81(8)	4.08 ± 0.49(8)
Statistical analysis				
Hydrocortisone effect	$P < 0.01$	$P > 0.05$	$P < 0.01$	$P < 0.01$
Diabetes	$P > 0.05$	$P < 0.05$	$P > 0.05$	$P < 0.01$
Interaction	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.01$

levels in diabetic animals are completely insensitive to hydrocortisone. Besides, it can be seen from table 3 that in intact fasted rats hydrocortisone injection results in a significant increase of blood sugar levels after 9 h and of blood insulin levels already detectable after 4.5 h.

5. Discussion

The failure of hydrocortisone to induce net synthesis of muscle glycogen in alloxan diabetic rats indicates that its action is mediated by insulin secretion. This is confirmed by the demonstration that blood concentration of insulin is increased after hydrocortisone administration before the muscle glycogen increase.

The rise in the insulin level might be related to the increase of blood sugar level [10,11] induced by hydrocortisone through stimulation of liver gluconeogenesis [12]. However it is possible also on the basis of the early increase in insulinemia, that this is due to direct stimulation of the hormone on endocrine pancreas (cfr. [5]).

Thus the effect of hydrocortisone on muscle gly-

Table 3
Effect of hydrocortisone administration (5 mg/rat) on the glycemic and insulinemic levels in the intact rat.

Hours following treatment	0	4.5	9	13	
Blood sugar (mg/ml)	0.97 ± 0.10(3)	0.97 ± 0.05(3)	1.23 ± 0.03(3)	1.23 ± 0.02(3)	$P < 0.01$
Blood insulin (μU/100 ml)	15.1 ± 0.42(3)	22.3 ± 0.99(3)	32.2 ± 2.60(3)	26.2 ± 0.17(3)	$P < 0.01$

cogen content appears to be dependent on (i) direct or indirect stimulation of insulin secretion; (ii) potentiation of the action of insulin by an independent increase of blood glucose which is the main substrate utilized by muscle for glycogen synthesis [13].

This interpretation is in line with the recent demonstration that also stimulation of liver glycogen synthesis by hydrocortisone is abolished in the diabetic animal [5] although the latter still shows increase of liver Glc-6-P [5] and of Glc-6-Pase [14].

The lag period observed for the effect of hydrocortisone on muscle glycogen probably corresponds to that necessary for the stimulation of liver gluconeogenesis and insulin secretion, as the lag period of insulin on glycogen synthesis in muscle is relatively short [15].

The present results do not imply that the mediation by insulin is a general mechanism by which corticosteroids act on muscle. In fact both on diaphragm *in vitro* [16,17] and on cell-free systems [18] it has been ascertained that corticosteroids show an early action on protein synthesis which is opposite to that of insulin.

Acknowledgement

This work was supported by grants from C.N.R. and Ministero della Pubblica Istruzione.

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