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GLUCOCORTICOID FUNCTION AND CYTOPLASMIC DEHYDROGENASE ACTIVITY OF THE ADRENALS FOLLOWING MECHANICAL AND TOXIC INJURY TO THE LIVER

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A decrease in the rate of corticosterone synthesis by adrenal slices and a decrease in cytoplasmic dehydrogenase activity of the adrenal cells were observed in male rats 48 h after partial hepatectomy, when the level of steroid metabolism in the liver was low, compared with the situation in animals undergoing a mock operation. The changes found are the result of depression of central mechanisms of stress as a result of the lowered steroid metabolism. Intraperitoneal injection of CCl_4 into rats in a dose of 0.1 ml/100 g body weight at these same times led to a marked increase in steroid production in the adrenal tissue and activation of NAD-dependent cytoplasmic dehydrogenases. The role of toxic damage to the glands in the changes in the functional state of the adrenocortical cells is discussed.

KEY WORDS: CCl_4; partial hepatectomy; adrenals; dehydrogenases; corticosteroids.

Acute and chronic liver diseases are accompanied by changes in adrenal function [6, 7]. However, the problem of whether these changes are the results of indirect inhibition of the adrenal glands through the depression of steroid metabolism or the result of direct toxic injury to the adrenal cortex still remains unsolved [9].

In this investigation a comparative analysis was made of the steroid producing function of the adrenals and of certain mechanisms of its energy provision in animals with mechanical and toxic liver damage.

EXPERIMENTAL METHOD

Experiments were carried out on four groups of noninbred male albino rats weighing 150-200 g. Under ether anesthesia laparotomy was performed on the animals of group 1, partial hepatectomy by the method of Higgins and Anderson on the rats of group 2, the rats of group 3 received an intraperitoneal injection of CCl_4 in a dose of 0.1 ml/100 body weight, and the rats of group 4 acted as the control. All manipulations were carried out between 9 and 11 a.m. The animals were decapitated 48 h later. The adrenals were removed, freed from connective tissue in the cold, cut into four parts, and incubated in a Warburg apparatus in Krebs–Ringer-phosphate buffer with 20 mM glucose at 37°C in an atmosphere of O_2. The concentration of 11-hydroxycorticosteroids (11-HCS) in the plasma and incubation samples was determined fluorometrically [12]. The trans-

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TABLE 1. Characteristics of Hypothalamic-Pituitary-Adrenal System 48 h after Experimental Procedures

Index studied	Intact control (11)	Mock operation (11)	Partial hepatectomy (11)	Poisoning with CCl_4 (10)
Relative weight of liver, g/100 g body weight	3,32±0,11	3,70±0,08*	2,37±0,09*	4,89±0,20*
Corticosterone metabolism, μg/100 mg liver/h	148,5±8,2	137,1±11,7	90,4±1,7*	60,7±13,9*
Corticosterone metabolism, μg/100 g body weight /h	4,81±0,37	4,97±0,42	2,11±0,21*	2,72±0,58*
Transcortin binding power	32,1±1,0	12,7±0,8*	15,2±1,7*	17,5±2,8*
Plasma 11-HCS concentration, μg %	31,6±2,8	27,4±3,6	28,9±0,4	34,1±3,5
Relative weight of thymus, mg/100 g body weight	99,2±9.0	75,7±3,5*	69,0±5,5*	78,9±9,3*

Legend. Here and in Table 2, results differing significantly from the control are marked by an asterisk ($P < 0.05$); number of animals shown in parentheses.

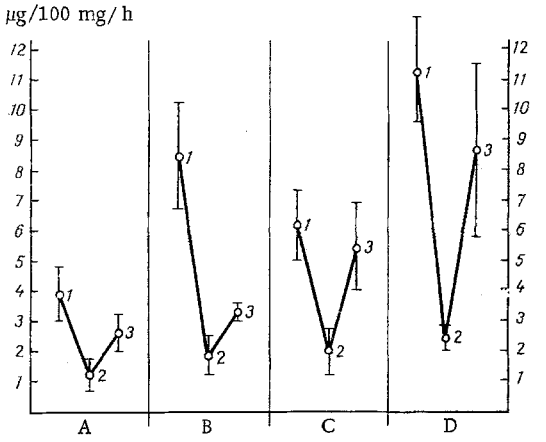

Fig. 1. Rate of corticosterone synthesis by adrenal slices during incubation. A) Intact control; B) 48 h after mock operation; C) 48 h after partial hepatectomy; D) 48 h after injection of CCl_4. 1) First hour of incubation; 2) 2nd hour of incubation after change of medium; 3) reaction to ACTH (1.5 i.u./100 mg). Confidence limits calculated for $P \leq 0.05$.

TABLE 2. Activity of Cytoplasmic Dehydrogenases of Adrenal Cells (10^{-4} M NADPH/mg protein/min)

Index studied	Intact control (9)	Mock operation (9)	Partial hepatectomy (9)	Poisoning with CCl_4 (12)
LD	384,0±15,45	190,5±18,9*	258,9±21,72*	1125,4±216,2*
MD	286,7±14,73	137,4±15,21*	212,4±50,46	1146,5±190,3*
G6PD + 6PGD	1229,0±54,91	1052,9±32,11*	851,8±104,3*	1121,6±178,4
ID	176,2±6,37	108,1±9,33*	137,8±16,42*	218,4±40.23
Protein, mg/100 mg tissue	6,6±0,3 $n=4$	7,36±1,12 $n=4$	11,0±2,88 $n=4$	7,26±2,3 $n=4$

cortin binding power was studied by Golikov's method. The rate of corticosteroid metabolism was determined by studying the disappearance of corticosterone during incubation of 100 μl of a 20% liver homogenate for 10 min at 37°C in a medium containing 15.1 mM KCl, 1.06 mM Tris, 1 μM NADPH, and 10 μg corticosterone. The reaction was stopped with cold 10% TCA. The fraction of adrenal hyaloplasm was obtained by differential centrifugation of the homogenates in a medium of KCl (150 mM) + Tris–HCl (10 mM). Activity of lactate dehydrogenase (LD) [3], malate dehydrogenase (MD) [4], glucose-6-phosphate dehydrogenase (G6PD), 6-phosphogluconate dehydrogenase (6-PGD) [15], and of NADP-dependent isocitrate dehydrogenase (ID) [8] was determined fluorometrically.

EXPERIMENTAL RESULTS

A comparative study of the glucocorticoid status of the rats with different types of liver damage gave the following results. 1) Partial hepatectomy, like injection of CCl_4, causes a decrease in glucocorticoid metabolism in the liver which is still present after 48 h. 2) Both procedures caused a sharp decrease in transcortin binding power. 3) The relative weight of the thymus was reduced, reflecting acute operative stress. 4) No significant changes in the plasma 11-HCS concentration was present after 48 h, indicating that the changes observed were compensated by that time (Table 1). The possible mechanism of this compensation is through feedback in the system controlling corticosteroid homeostasis [2, 11].

Investigations of the rate of corticosterone synthesis by adrenal slices in course of incubation (Fig. 1) revealed an increase in secretion during the first hour of incubation of material from rats undergoing the mock operation, possibly as a result of operative stress. In the partially hepatectomized rats a decrease in the rate of corticosterone synthesis was found during the first hour of incubation by comparison with rats undergoing the mock operation, indirect evidence of a decrease in the secretion of endogenous ACTH in the animals of this group. This conclusion is confirmed by the higher response of the animals of this group to exogenous ACTH than of the control group and of the rats undergoing the mock operation.

Despite the extension of the operation producing stress, the steroid-producing reaction of the adrenals was less marked in the partially hepatectomized rats. The most probable reason for the special feature of stress in this case is a decrease in the intensity of steroid metabolism compared with animals undergoing the mock operation.

Steroid production in the animals with toxic injury to the liver followed a different pattern. The writers previously showed an increase in the plasma 11-HCS concentration 24 and 28 h after administration of a similar dose of CCl_4 to Wistar rats. Meanwhile a sharp decrease was found in the response of the hypothalamic-pituitary-adrenal system to administration of ACTH to rats [6]. Even more unexpected was the increase in the rate of corticosterone synthesis in the experiments in vitro at these times. A marked increase in the rate of steroid biosynthesis and of the reaction to exogenous ACTH in vitro was observed 48 h after injection of CCl_4 (Fig. 1D). One cause of the change in the mechanisms of steroid production could be a change in the state of the cells of the adrenal cortex.

Measurement of adrenal cytoplasmic dehydrogenase activity (Table 2) showed that it was reduced in animals undergoing both the mock operation and hepatectomy. This dynamics of enzyme activity may reflect the late phases of the post-stress period [5]. What is important is that the decrease in dehydrogenase activity was less marked after partial hepatectomy, confirming the earlier conclusion that the response of steroid production to stress in the animals of this group was of lower intensity.

After administration of CCl_4, on the other hand, marked activation of NAD-dependent dehydrogenases (LD and MD) was observed and activity of the NADP-dependent enzymes remained high. Steroid production is known to be closely linked with the activity of the final stages of glycolysis [14] and of the pentose phosphate shunt [13], the power of which is determined by G6PD and 6PGD activity. Meanwhile activity of the NAD-dependent energy-synthesizing reactions of the cytoplasm reflects the intensity of structural syntheses in the cell [10]. The distinguishing features of the enzyme spectrum of the adrenal cells of rats poisoned with CCl_4 probably reflect their special plastic state as a rssult of toxic injury to the glands [9]. Isolated activation of NAD-dependent dehydrogenases combined with residual high activity of NADPH-generating enzymes evidently creates a favorable basis for stimulation of steroid-synthesizing reactions of the adrenals.

The lower intensity of stress following injury to the liver than after the mock operation thus has one distinctive feature which depends on the mode of injury. After partial hepatectomy this effect can be observed at the level of the adrenal glands, unlike after toxic injury by CCl_4, when depression of steroid production in the adrenals is not observed. The antistressor effects associated with different types of injury may thus arise at the organ or system level.

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GLUCOSE TRANSPORT IN THE SMALL INTESTINE AFTER LIGATION OF THE BILIARY-PANCREATIC DUCT IN RATS

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Muco-serosal glucose transport along and against the concentration gradient (the modified "everted sac" method) was determined in six segments of small intestine of rats on the 4th, 7th, 14th and 28th days after ligation of the combined biliary-pancreatic duct. The dynamics of modification to the transport systems in each segment of the intestine differed according to their dependence on the sources of energy. In the early period after the operation transport along the concentration gradient was mainly intensified, but in the later stages transport against this gradient was intensified.

KEY WORDS: small intestine; glucose transport; ligation of biliary-pancreatic duct.

Adaptive and compensatory reactions in different physiological systems evidently obey certain general rules [5, 7, 10]. Attempts have recently been made to extend ideas regarding methods of regulation to the metabolic level [1, 2]. These views must be taken into consideration when adaptations in systems of membrane hydrolysis and transport are studied, for the metabolic properties of the enterocytes vary along the course of the small intestine [3, 8, 11]. Glucose transport in enterocytes is known to take place by several mechanisms [6, 12, 13]. With different dietary intakes the properties of the transport system may vary differently along (TCG) or against (TACG) the concentration gradients. No comparison between these two systems of transport under pathological conditions has been carried out until recently. Ugolev [8] has postulated that against the background of adaptive and compensatory structural changes in the small intestine the properties of transport systems characterized by unequal dependence on sources of energy may vary differently.

In this investigation this problem was studied in relation to glucose by comparing TCG and TACG in all parts of the small intestine of rats after ligation of the biliary-pancreatic duct.

EXPERIMENTAL METHOD

Experiments were carried out on 25 adult albino rats kept on a mixed diet and starved for 18 h before being used. The rats were decapitated on the 4th-28th day after ligation of the biliary-pancreatic duct; the small intestine (without the duodenum) was removed and divided into six equal segments. Muco-serosal glucose transport was studied by a modified "everted sac" method [4, 12]. Two lengths (about 5 cm) were iso-

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