EFFECT OF HYDROCORTISONE, DEOXYCORTICOSTERONE ACETATE, TESTOSTERONE PROPIONATE, AND ESTRADIOL DIPROPIONATE ON INCORPORATION OF GLYCINE-1-C^{14} INTO SUBCELLULAR FRACTIONS OF LIVER CELLS

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Hydrocortisone considerably increased protein synthesis in the microsomal fraction (as reflected by incorporation of glycine-1-C^{14}) in experiments on rats. Testosterone propionate increased protein biosynthesis in this fraction to a lesser degree. Estradiol dipropionate affected protein synthesis in the microsomal fraction in two phases: it inhibited incorporation of the label into proteins 2 h after administration, and stimulated it 12 h after administration. Deoxycorticosterone acetate had the opposite action to hydrocortisone.

The steroid hormones can modify protein biosynthesis in the liver cells through their action on the functional state of the genome [1, 2, 4].

The object of the present investigation was to study the effect of a glucocorticoid, for which the liver is the target organ, and also of a mineralocorticoid, androgen, and estrogen on the kinetics of incorporation of a labeled amino acid into proteins of the subcellular fractions of the hepatocytes.

EXPERIMENTAL METHOD

Experiments were carried out on 288 male albino rats weighing 180-220 g. The animals were fasted for 12 h before the experiment.

Hydrocortisone (series I), deoxycorticosterone acetate (series II), testosterone propionate (series III), and estradiol dipropionate (series IV) were injected intraperitoneally in a dose of 10 mg/kg body weight 2, 4, 6, 12, 24, and 48 h before sacrifice.

The degree of protein synthesis was estimated from the incorporation of labeled amino acid (glycine-1-C^{14}) into the nuclear, mitochondrial, and microsomal fractions and also into the postmicrosomal supernatant. Glycine was injected intraveneously in a dose of 0.5 μCi/g body weight 2 h before sacrifice. All procedures for isolation of the subcellular fractions were carried out in the cold. The rats were decapitated, and the thorax and abdomen opened quickly. The liver was washed with 0.14 M sodium chloride solution at 4°C, and then removed, dried on filter paper, and cut into samples weighing 1 g, which were homogenized in a Potter's homogenizer (with Teflon pestle) with 10 ml 0.25 M sucrose solution. The homogenate was filtered through two layers of gauze, after which the nuclei were separated at 800 g for 10 min. The supernatant was removed and centrifuged at 10,000 g for 20 min to separate the mitochondria. Next, by centrifugation at 105,000 g for 60 min, microsomes were obtained and resuspended in 5 ml 0.25 M sucrose solution, and again sedimented. The nuclear and mitochondrial fractions were washed in sucrose 4 times. Protein was precipitated from all fractions by 10% TCA solution. It was then washed several times

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TABLE 1. Effect of 10 mg/kg Hydrocortisone, Deoxycorticosterone Acetate, Testosterone Propionate, and Estradiol Dipropionate on Incorporation of Glycine-1-C^{14} into Protein of Subcellular Fractions of Hepatocytes (M ± m)

Hormone	No. of animals	Fraction	Incorporation of glycine at different times after injection of hormone, in % of control					
			2 h	4 h	6 h	12 h	24 h	48 h
Hydrocortisone	72	Nuclei	68 5± 5,1*	95,2± 6,8	107,3± 6,1	159,6± 8,7 *	132,8± 6,4 *	104,7± 5,8
		Mitochondria	49,6± 4,2 *	92,9± 5,4	127,8± 6,9 *	93,1± 7,4	89,4± 6,8	106,7± 7,2
		Microsomes	149,7± 6,8 *	138,5± 7,1 *	140,8± 8,9 *	254,9± 9,8 *	125,3± 7,5 *	105,4± 6,3
		Supernatant (105,000 g)	138,4± 6,5 *	142,6± 7,2 *	168,3± 8,7 *	211,7± 9,6 *	163,2± 8,3 *	98,7± 5,4
Deoxycorticosterone acetate	72	Nuclei	119,4± 8,7	75,1± 5,8 *	67,7± 5,0 *	55,2± 4,5 *	72,9± 5,6 *	102,5± 6,7
		Mitochondria	148,7± 6,6 *	137,3± 6,1 *	91,2± 7,2	108,6± 6,9	97,1± 5,9	101,8± 6,2
		Microsomes	88,2± 8,2	79,2± 5,2 *	71,1± 4,7 *	53,1± 4,1 *	112,3± 6,8	99,3± 5,7
		Supernatant (105,000 g)	92,3± 5,9	74,8± 5,3 *	68,4± 6,5 *	54,7± 4,3 *	89,5± 7,4	107,2± 7,5
Testosterone propionate	72	Nuclei	78,5± 4,8 *	82,4± 6,5	92,4± 5,1	77,5± 4,7 *	117,0± 8,7	103,1± 7,6
		Mitochondria	72,5± 4,6 *	95,6± 5,7	129,3± 4,3 *	105,9± 5,3	58,3± 4,4 *	96,6± 6,1
		Microsomes	128,1± 5,3 *	121,1± 4,5 *	134,5± 5,6 *	143,7± 7,4 *	136,4± 6,2 *	108,3± 6,5
		Supernatant (105,000 g)	119,6± 6,2	128,4± 5,4 *	130,9± 5,8*	143,5± 6,7 *	148,7± 7,0 *	110,5± 8,4
Estradiol dipropionate	72	Nuclei	78,1± 5,1 *	89,7± 6,3	103,4± 5,2	100,2± 5,8	92,8± 6,3	104,1± 5,2
		Mitochondria	77,2± 4,9 *	102,4± 5,8	110,1± 6,8	125,3± 5,1 *	105,2± 7,6	98,3± 6,4
		Microsomes	57,8± 3,8 *	98,5± 5,6	111,3± 5,9	127,7± 5,2 *	91,9± 5,9	95,4± 4,7
		Supernatant (105,000 g)	68,3± 4,7 *	109,2± 6,9	107,5± 7,3	135,4± 5,5 *	132,6± 6,7 *	103,6± 6,0

* Difference from control statistically significant.

with 5% TCA solution and dried for 12 h. The radioactivity of specimens from 10-mg samples was determined on a T-25-BFL end-window type counter with PST-100 scaler and expressed in pulses/min/mg protein.

EXPERIMENTAL RESULTS

The results (Table 1) show that steroids of the various classes differ in their effects on incorporation of glycine-1-C^{14} into proteins of the subcellular fractions of the liver cells.

Under normal conditions, more amino acid was incorporated into the microsomal fraction and postmicrosomal supernatant, less into the nuclear fraction, and less still into the mitochondria.

The glucocorticoid had a biphasic action on incorporation of the label into the nuclei and mitochondria of the hepatocytes: in the early stages it inhibited, but later it stimulated incorporation of the isotope. Incorporation of the precursor into the microsomal fraction was significantly increased 2 h after administration of hydrocortisone.

As Table 1 shows, deoxycorticosterone acetate had the opposite action.

Testosterone propionate acted in the same direction as hydrocortisone, but its effect was less marked.

Estradiol dipropionate had a biphasic action on incorporation of the labeled amino acid into protein of the microsomal fraction: it inhibited protein synthesis 2 h after injection, but stimulated it 12 h after injection.

These results are confirmed by the observations of other workers [3, 5] who found that aggregation of the ribosomes with the formation of polysomes takes place after administration of glucocorticoids, indicating the intensification of protein biosynthesis in the liver cells.

Analysis of these results suggests that the less marked effect of the androgen and estrogen, as well as the opposite effects of the mineralocorticoid and glucocorticoid, are connected with the fact that the liver is the target organ only for hydrocortisone.

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