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INHIBITION BY ADENOSINE OF THE CORTISOL-INDUCED LIVER GLYCOGEN ACCUMULATION IN ADRENALECTOMIZED RATS

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Abstract—The intraperitoneal administration of adenosine to adrenalectomized rats prevents the cortisol accumulation of liver glycogen. Adenosine inhibits the cortisol-induced uptake of [^{14}C]glucose and [^{14}C]alanine into liver glycogen by approximately 90 per cent. However, the specific activity of liver glycogen found in animals receiving adenosine or adenosine plus cortisol and radioactive glucose or alanine is higher than that observed in animals injected with saline or cortisol. The lipogenic process of the epididymal fat pad is also stimulated *in vivo* by adenosine. In rats injected simultaneously with adenosine plus cortisol, it was found that the incorporation of radioactive glucose into the epididymal fat pad predominates over the cortisol-induced incorporation of glucose into liver glycogen. The suggestion is made that the lipogenic effect of adenosine causes a marked increase in the demand of glucose by the adipose tissue which results in the following set of events: enhanced glycogenolysis, a faster glycogen turnover, and a low level of liver glycogen.

SEVERAL substances capable of inhibiting gluconeogenesis have been used in the study of the metabolic regulation of this pathway. L-Tryptophan,[1] ethanol,[2,3] biguanides[4] and (+)-acylcarnitine[5] inhibit at specific steps either the production of glucose or the deposition of glycogen. The intraperitoneal injection of nonspecific yeast ribonucleic acid (RNA) or an equimolecular mixture of adenine and guanine nucleotides also inhibits the cortisol-induced accumulation of liver glycogen in adrenalectomized rats.[6]

The inhibition of the cortisol-induced glycogen deposition by adenine and guanine nucleotides and their hydrolysis products was used to study certain aspects of glycogen metabolism. Since it is probable that the gluconeogenic action of glucocorticoids could partially be because of their permissive effect on lipolysis in adipose tissue,[7] part of the presently reported study has been extended to the effect of adenosine on the lipid metabolism of peripheral adipose tissue. Dole has described a lipogenic and antilipolytic action of AMP and adenosine *in vitro* on the epididymal fat pad,[8] and a role of the fatty acid oxidation on the control of hepatic gluconeogenesis has also been proposed.[9]

EXPERIMENTAL

The experiments were performed on male Wistar rats weighing 120–150 g that had been adrenalectomized 72 hr prior to the experiments. The adrenalectomized animals were not fed for a period of 5 hr before the beginning of the experiments: this short starvation period was chosen since the animals injected with adenosine frequently die

in severe hypoglycemia with a longer starvation time. Although the animals were not subjected to strict feeding and lighting schedules, all of them were kept under the same conditions; after adrenalectomy the animals were maintained in separate cages and drinking water was substituted by an 0·85% NaCl solution; the day of the experiment the food was removed at 7 a.m. and control and experimental rats were sacrificed alternately by decapitation and exsanguination. The liver and muscle glycogens were assayed according to the method of Hassid and Abraham.[10] Protein was determined by the method of Lowry *et al.*[11] Blood glucose was estimated by the method of Nelson.[12] Lipogenesis was considered as equivalent to the incorporation of [UL-^{14}C]-glucose into organic solvents extractable material, and the latter was assayed according to the technique of Folch *et al.*[13] Glycogen radioactivity was counted in Bray's solution[14] and that of lipids in toluene solution of 2,5-diphenyloxazole and 2,2-*p*-phenylenebis (5-phenyloxazole). A Packard Tri-Carb liquid scintillation spectrometer was used for all radioactive measurements.

The purine and pyrimidine nucleotides and their hydrolysis products were obtained from Nutritional Biochemical, U.S.A.; cortisol hemisuccinate was a gift from Upjohn, S.A. de México; [UL-^{14}C-D]glucose, with a specific activity of 257 mc/mM, was obtained from International Chemical and Nuclear Corp.; [UL-^{14}C-L]alanine, with a specific activity of 105 mc/mM, was purchased from Calbiochem.

RESULTS AND DISCUSSION

Experiments were conducted to identify a possible component(s) which could reproduce the RNA-induced inhibition of the cortisol-mediated liver glycogen accumulation.[6] A marked inhibition of the cortisol-induced process was observed with adenosine; AMP was less effective, and other purine and pyrimidine nucleotides had no effect (Table 1).

Cortisol strikingly increased the total glycogen radioactivity in the liver when either [^{14}C]glucose or [^{14}C]alanine is given; however, its specific activity was lower than that of the control animals (Table 2). In the control rats and those treated with adenosine, the net labelling of hepatic glycogen with [^{14}C]glucose or alanine was of a similar extent; nevertheless, a higher specific activity was observed in the adenosine-treated rats. When cortisol and adenosine were given simultaneously, the total glycogen radioactivity in the liver was similar to that of control or adenosine-injected rats, but its specific activity was increased.

The incorporation of uniformly labeled glucose into the hind leg muscle glycogen under the influence of adenosine with or without cortisol is also presented in Table 2. It may be observed that adenosine augments the specific activity of muscle glycogen regardless of the presence of cortisol. The results, although statistically nonsignificant, suggest that adenosine increases the uptake of glucose by the muscular mass. It should be recalled in this respect that the muscular mass represents 50 per cent of the body weight.

The concentration of blood glucose at the end of the experimental period was measured in rats previously injected with adenosine, cortisol or adenosine plus cortisol. Blood glucose decreased from 53·5 $\pm$ 4·7 (13 determinations) to 41·2 $\pm$ 4·0 mg/100 ml (six determinations) after adenosine treatment; cortisol elevated the blood glucose level to 75·0 $\pm$ 6·1 mg/100 ml (15 determinations), and with adenosine plus cortiso

TABLE 1. EFFECT OF SOME HYDROLYSIS PRODUCTS OF RIBONUCLEIC ACID ON THE ACCUMULATION OF LIVER GLYCOGEN INDUCED BY CORTISOL*

Injected compound	Liver glycogen (mg/g wet weight)† Mean ± S.E.	No. of determinations
Saline	0·50 ± 0·05	31
Cortisol	10·89 ± 1·01	30
Cortisol + AMP	2·94 ± 0·80‡	7
Cortisol + GMP	6·40 ± 2·34	7
Cortisol + CMP	9·94 ± 3·24	4
Cortisol + UMP	6·84 ± 1·78	5
Cortisol + adenosine	0·64 ± 0·12‡	16
Cortisol + guanosine	6·58 ± 2·10	6
Cortisol + adenine	6·86 ± 1·6	5
Cortisol + ribose	11·04 ± 2·82	6
Adenosine	0·47 ± 0·09	20

* Adenosine suspended in saline at pH 7·3 was given by intraperitoneal route at a dose of 200 mg/kg. The other nucleotides derivatives were given similarly in a dose equimolar to that of adenosine. Cortisol was injected at a dose of 25 mg/kg separately but simultaneously to the tested substance. The experimental animals were sacrificed 3·5 hr after the administration of the substances.

† In different experimental groups the amount of liver protein was very similar.

‡ Cortisol *vs.* cortisol + adenosine, $P < 0.01$. Cortisol *vs.* cortisol + AMP, $P < 0.02$.

TABLE 2. EFFECT OF ADENOSINE AND/OR CORTISOL ON THE INCORPORATION OF RADIOACTIVE PRECURSORS INTO LIVER AND MUSCLE GLYCOGEN OF ADRENALECTOMIZED RATS*

	[UL-^{14}C]alanine		[UL-^{14}C-glucose]		
	dis./min × 10^3	dis./min × 10^3	dis./min × 10^3	dis./min × 10^3	dis./min × 10^3
Experimental substance	total liver	mg of liver glycogen	total liver	mg of liver glycogen	mg of muscle glycogen
Saline	8·0 ± 1·2 (6)	3·8 ± 1·2 (6)	9·3 ± 0·2 (5)	4·2 ± 0·6 (5)	0·14 ± 0·02 (5)
Cortisol	77·1 ± 1·2 (5)	2·9 ± 0·6 (5)	73·2 ± 20·4 (5)	1·5 ± 0·2 (5)	0·12 ± 0·02 (5)
Adenosine	7·8 ± 1·6 (5)	5·9 ± 0·6 (5)	9·4 ± 0·5 (8)	6·7 ± 0·5 (8)	0·32 ± 0·07 (4)
Adenosine + cortisol	7·8 ± 1·6 (5)	7·4 ± 0·7 (5)	12·9 ± 4·0 (8)	9·2 ± 1·5 (8)	0·28 ± 0·11 (5)

* Each figure represents the value of the mean ± standard error. Numbers in parenthesis represent the number of experimental animals. The experimental conditions were the same as in Table 1; radioactive alanine or glucose was given separately but simultaneously with the experimental substance at a dose of 50 μc/kg. Muscle glycogen was determined in the hind leg muscle.

a value of 68·6 ± 3·3 (six determinations) was obtained. In other words, adenosine tended to cause a discreet hypoglycemia whether or not cortisol was present.

As a lipogenic and antilipolytic effect *in vitro* of adenosine on the epididymal fat pad has been reported,[8] it is possible that blood glucose may be converted to fat in adipose tissue. The results in Table 3 indicate that adenosine stimulated the lipogenic process in adipose tissue regardless of the administration of cortisol. Considering that the total peripheral adipose tissue corresponded to about 15 per cent of the body weight,[15] the administration of adenosine should increase significantly the demand of glucose.

TABLE 3. EFFECT OF CORTISOL AND/OR ADENOSINE ON THE INCORPORATION OF [UL-^{14}C]GLUCOSE INTO LIPIDS OF DIFFERENT TISSUES IN ADRENALECTOMIZED RATS*

Experimental substance	Tissue			
	Brain	Liver	Epididymal fat pad	Subcutaneous adipose tissue
	(dis./min/mg of lipids) (Mean ± S.E.)			
Saline	133·2 ± 20·6 (6)†	157·2 ± 16·8 (6)	47·1 ± 16·4 (6)	19·9 ± 2·9 (11)
Cortisol	125·0 ± 12·6 (6)	183·9 ± 15·8 (6)	40·1 ± 2·6 (6)	19·6 ± 2·6 (9)
Adenosine	101·5 ± 18·0 (6)	175·8 ± 12·8 (8)	122·5 ± 23·0‡ (8)	37·0 ± 2·7‡ (9)
Adenosine + cortisol	148·0 ± 15·2 (5)	213·8 ± 23·0 (8)	111·8 ± 18·3‡ (7)	33·2 ± 4·5‡ (9)

* Experimental conditions were the same as in Table 1 and 2.
† Numbers in parentheses represent number of experimental animals.
‡ Saline v. adenosine, $P < 0{\cdot}01$ in epididymal fat pad and in subcutaneous adipose tissue. Cortisol *v.* adenosine + cortisol, $P < 0{\cdot}01$ in epididymal fat, $P < 0{\cdot}02$ in subcutaneous adipose tissue.

Adenosine also produced a striking increase in the conversion of radioactive glucose to lipids of the epididymal fat pad. On the other hand, the incorporation of [UL-^{14}C]glucose into the lipids of liver and brain was not significantly affected by the hormone or by the nucleoside (Table 3).

The hypoglycemia and the increased incorporation of glucose into fat and into muscle glycogen provoked by adenosine raised the question as to whether the metabolic effects are directly induced by adenosine or mediated by insulin release. The following observations suggest that the former possibility seems to be more feasible: Adenosine shows a lipogenic action on the epididymal fat pad *in vitro* in the absence of insulin,[8] and the lipogenic action of adenosine also exists in alloxan diabetic rats which have high blood glucose levels.* However, with the results presented in this paper, the participation of insulin in certain metabolic effects of adenosine cannot be definitively discarded.

The incorporation of [UL-^{14}C]glucose into liver glycogen and epididymal fat pad in the 4 experimental groups studied as a function of time is shown in Fig. 1. Adrenalectomized rats injected with saline showed a discreet uptake of radioactive glucose

*Unpublished data.

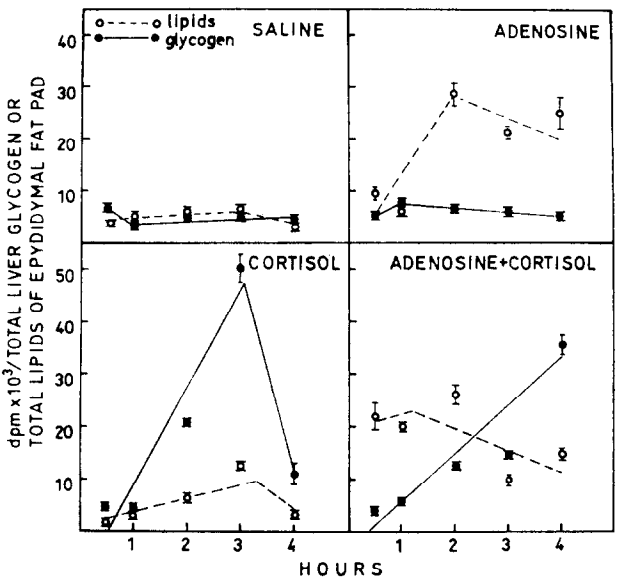

FIG. 1. Incorporation of [UL-^{14}C]glucose into liver glycogen and epididymal fat pad as a function of time. Radioactive glucose at a dose of 20 μc/kg was given at time 0, separately but simultaneously to the experimental substance(s). The animals were sacrificed at the indicated time. Experimental mean values ± standard error are given for each experimental group (four to five animals). The slopes (± standard error) corresponding to the linear regression for the individual values of total [^{14}C]glucose incorporated into liver glycogen and epididymal fat pad were calculated by the method of "least squares". Slopes of the incorporation of [UL-^{14}C]glucose into liver glycogen—Saline: −5·81 ± 0·42, 0·36 ± 0·07; cortisol: 18·32 ± 0·60, −39·4 ± 3·46; adenosine: 6·22 ± 0·76, −0·86 ± 0·07; adenosine + cortisol: 8·36 ± 0·31. Slopes of the incorporation of [UL-^{14}C]glucose into total lipids of epididymal fat—Saline: 0·76 ± 0·18, −2·0 ± 0·67; cortisol: 4·02 ± 0·28, −8·52 ± 0·92; adenosine: 14·59 ± 1·49, −4·07 ± 1·52; adenosine + cortisol: 1·98 ± 1·69, −4·05 ± 0·92.

into liver glycogen and epididymal fat pad, but after a lag period of 1 hr, cortisol activated the incorporation of glucose into liver glycogen while adenosine, in the absence of cortisol, stimulated the conversion of glucose into lipids. The simultaneous administration of adenosine and cortisol resulted in a predominance of the lipogenic action of the nucleoside over the glucogenic effect of the hormone which was observed 30 min after the injection of the experimental substances; concomitantly, with a diminution in the lipogenic effect of the nucleoside was observed an increase in the incorporation of labeled glucose into liver glycogen.

The role of adenosine on the cortisol-mediated glycogen deposition in the liver could be the result of an inhibition in the glycogen biosynthesis stimulated by the steroid, or an increase in glycogen breakdown.

Several facts were in favor of the second alternative and/or against the first one: (1) Adenosine alone produced discreet stimulation in glycogen biosynthesis during the first hour of its administration (Fig. 1). (2) The specific activity of liver glycogen from adenosine-injected rats was higher than that of control animals (Table 2), while the total amount of liver glycogen remained unchanged (Table 1). (3) This situation was particularly clear in rats treated with adenosine and cortisol as compared with those receiving only cortisol; after the injection of the hormone the amount of hepatic

glycogen increased to 10·89 mg/g wet weight (Table 1) but this glycogen showed a low specific activity 1·5 dis./min $\times$ 10^3/mg of liver glycogen (Table 2); if adenosine was given together with cortisol, the amount of liver glycogen was only 0·64 mg/g wet weight (Table 1), but its specific activity was six times higher; i.e. 9·2 dis./min $\times$ 10^3/mg of liver glycogen (Table 2). In addition, it should be acknowledged that in rats treated with adenosine alone or with adenosine plus cortisol the radioactive glucose was being actively utilized by adipose tissue (Table 3); this would probably cause a diminution in the specific activity of blood glucose; nevertheless, the specific activity of the glycogen in these animals was high. (4) The increase in the levels of blood glucose normally observed after the enhancement of glycogenolysis might be obscured in adenosine-treated rats by the strong stimulation of glucose utilization by adipose and muscular tissues provoked by the nucleoside. (5) RNA which mimics the actions of adenosine,[6] injected with or without cortisol, produced an 8-fold increase in the activity of glycogen synthetase, which did not seem to be compatible with an inhibition in glycogen biosynthesis.

Although no precise measurements on the turnover and half-life of hepatic glycogen were made, the data obtained with the animals receiving adenosine and cortisol suggested an increase in glycogen turnover. The data presented did not conclude that the assumed increase in liver "glycogen turnover" after adenosine injection had an hepatic or extrahepatic origin. One possibility was that the lipogenic and glucogenic actions of adenosine in the fatty tissues and muscular mass respectively resulted in an increased demand of glucose by these tissues; this was reflected in the decrease of blood glucose and the hypoglycemia was followed by an enhancement in liver glycogenolysis.

Nevertheless, the potential conversion of adenosine into 3′,5′ cyclic AMP which will activate liver phosphorylase[16,17] and will produce an increase in glycogenolysis cannot be discarded. Such conversion might be related to the specificity of adenosine in producing the effects described in this paper.

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