ADENOSINE, A GLUCOGENIC AND LIPOGENIC COMPOUND

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1. Introduction

It has been reported that adenosine inhibits the liver glycogen accumulation induced by cortisol in adrenalectomized rats [1]. This inhibition results from an enhanced glycogenolysis that satisfies an increase in the demand of glucose, which is in turn provoked by adenosine in adipose and muscular tissues [1]. In the course of the experiments with adrenalectomized rats, it was observed that the injection of adenosine into normal animals resulted in a different response: an increase in the incorporation of UL-^{14}C-glucose into liver glycogen and epididymal fat pad simultaneous with a dramatical augmentation in the total liver glycogen turnover rate.

2. Materials and methods

The experiments were performed with male Wistar rats weighing between 120–150 g and fasted for 16 hr. The animals were not subjected to strict feeding and lighting schedules, but were maintained under very similar conditions, and control and experimental animals were sacrificed alternately by decapitation and exsanguination.

Glycogen content, glycogen radioactivity and lipogenesis were assayed as previously described [1]. Liver glycogen turnover was measured according to Shull and Meyer [2]. Only a few attempts to obtain a quantitative value of hepatic glycogen turnover have been reported [2, 3], and this is probably due to the difficulties in obtaining a real value. The main problems in obtaining this figure are discussed by Shull and Meyer [2]. In order to obtain the data of total liver glycogen turnover rate presented here, it was assumed that our experimental animals, being subject to the administration of the test substances, were similar in conditions of blood volume, liver weight, total body water, blood glucose, and liver glycogen at the beginning of the experiment.

3. Results and discussion

The results of the initial exploration of the nucleoside effect in normal rats were taken 3.5 hr after adenosine administration (fig. 1); the nucleoside promoted a 2-fold increase in the level of liver glycogen as found in cortisol-injected rats, and the simultaneous administration of adenosine and cortisol gave an additive response. As judged by the specific activity of the hepatic glycogen, adenosine alone produced a 13-fold increase in the incorporation of labelled glucose into liver glycogen; a similar 6-fold increase was observed with labelled alanine. On the other hand, cortisol provoked a 2.5-fold increase with either labelled precursors.

Since adenosine produced a striking increase in glycogen specific activity (either from labelled glucose or alanine), without a comparable enhancement in the amount of liver glycogen, the possibility of an increase in the turnover rate of the hepatic polysaccharides was considered. The results of total glycogen turnover are presented in fig. 2, the calculations being made according to Shull and Meyer [2]. In all of the studied groups, a maximum incorporation of ^{14}C-glucose into liver glycogen was observed 3 hr after the administration of the experimental substances, this being identical to the data reported for normal and obese hyper-

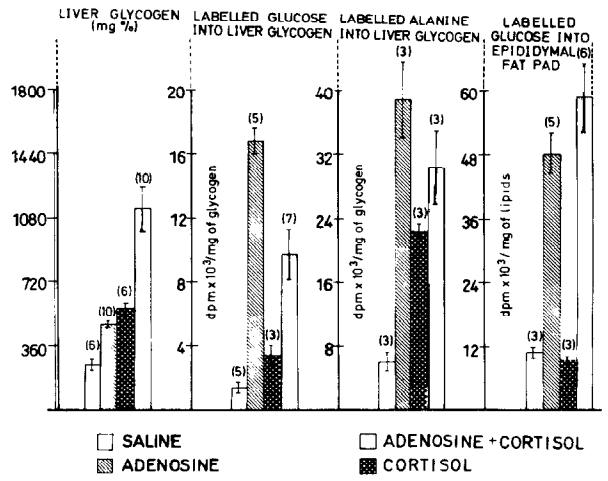

Fig. 1. Effect of adenosine on hepatic glycogen synthesis and lipogenesis in the epididymal fat pad. Adenosine suspended in saline at pH 7.3 was administered by an intraperitoneal route at a dose of 200 mg/kg of body weight. Cortisol was injected at a dose of 25 mg/kg and radioactive glucose or alanine at a dose of 50 μCi/kg. The substances were administered separately but simultaneously. Standard error is represented by the vertical lines. The number of rats used for each experiment is given in parenthesis.

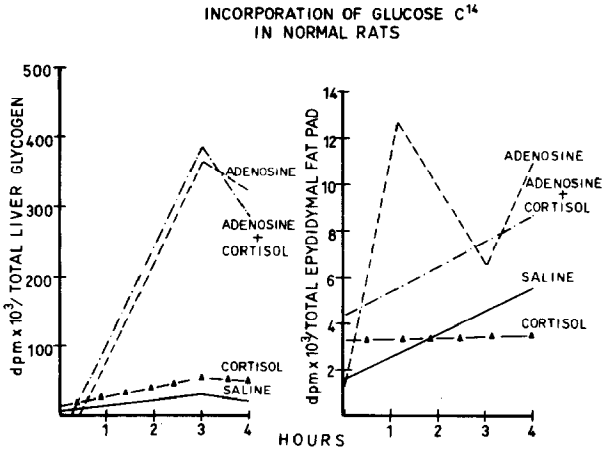

Fig. 2. Incorporation of UL-^{14}C-glucose into liver glycogen and epididymal fat pad as a function of time. Radioactive glucose at a dose of 20 μCi/kg was administered at time 0, separately but simultaneously with the experimental substance(s). The animals (5 for each experimental period) were sacrificed at 1, 2, 3 and 4 hr after the administration of the experimental substance(s). Other experimental conditions as in fig. 1. The slopes, (dpm/hr) ± standard error, correpond to the linear regression for the individual values of total ^{14}C-glucose incorporated into liver glycogen and epididymal fat pad and were calculated by the method of "least squares".

glycemic mice [2]. The positive slopes obtained with adenosine or adenosine-plus-cortisol groups are significantly different ($p < 0.001$) from the slopes of either saline or cortisol groups. A comparison of the slopes, calculated for the most probable straight line, demonstrates that the turnover of liver glycogen was approximately 23 times greater per total glycogen in the adenosine-injected rats than in their saline-injected controls. Cortisol did not enhance total liver glycogen turnover when injected into animals receiving adenosine. The tremendous increase in total liver glycogen turnover after adenosine injection points to this nucleoside as being the most powerful known compound acting on hepatic glycogen metabolism.

The *in vivo* lipogenic action of adenosine on the epididymal fat pad is also presented in fig. 1. This effect was similar to that observed in adrenalectomized rats [1], and to that described by Dole [4] in *in vitro* experiments: the nucleoside, alone or with cortisol, produces a 5-fold increase in the incorporation of ^{14}C-glucose into the epididymal fat pad. The time course incorporation of UL-^{14}C-glucose into the epididymal fat pad was studied in the same animals in which the glycogen turnover rate was measured (fig. 2). The *in vivo* lipogenic action of adenosine was detected scarcely 30 min after administration of the nucleoside, and a maximum effect was observed 1 hr after the injection of the substance. A second increment in lipogenesis was observed 3 hr later. The blocking of the effect of adenosine in the presence of exogenous cortisol as shown in the figure might be

Slopes (dpm × 10^3 hr) of the incorporation of UL-^{14}C-glucose into total liver glycogen:

Saline:	6.75 ± 0.66 and	−11.60 ± 4.47
Cortisol:	18.32 ± 2.20 and	−2.87 ± 1.60
Adenosine:	195.16 ± 14.64 and	−68.86 ± 14.90
Adenosine + cortisol:	149.67 ± 5.78 and	−149.26 ± 28.20

Slopes (dpm × 10^3 hr) of the incorporation of UL-^{14}C-glucose into total lipids of epididymal fat.

Saline:	1.17 ± 0.08
Cortisol:	−0.14 ± 0.12
Adenosine:	11.60 ± 1.30 and −3.11 ± 0.12
	4.78 ± 0.60
Adenosine + cortisol:	1.09 ± 0.11

Table 1
Influence of nucleotide derivatives in normal rats on glycogen synthesis in the liver and lipogenesis in the epididymal fat pad.

Substance	Liver glycogen (mg %)	Glycogen (dpm × 10^3/mg)	Lipids (dpm × 10^3/mg)
Saline	0.85 ± 0.12 (20)	1.06 ± 0.24 (20)	10.0 ± 1.7 (22)
NaH_2PO_4	1.40 ± 0.11 (3)	0.18 ± 0.02 (3)	5.6 ± 1.5 (3)
Ribose	0.90 ± 0.19 (3)	0.15 ± 0.06 (3)	6.5 ± 2.3 (3)
Adenine	1.57 ± 0.67 (4)	0.14 ± 0.05 (4)	8.8 ± 2.8 (4)
Adenosine	1.53 ± 0.31 (15)	5.37 ± 1.16 (15)	34.1 ± 6.1 (17)
Adenylic acid	1.38 ± 0.44 (3)	2.67 ± 1.00 (3)	15.3 ± 2.6 (4)
Guanosine	0.82 ± 0.31 (10)	2.15 ± 0.76 (10)	24.3 ± 5.2 (10)
Inosine	0.31 ± 0.11 (9)	5.71 ± 1.28 (9)	22.9 ± 5.8 (6)
Uridine	0.76 ± 0.16 (11)	1.08 ± 0.69 (11)	17.6 ± 3.2 (6)
Citidine	0.47 ± 0.13 (9)	1.79 ± 0.53 (9)	11.8 ± 2.3 (10)
Timidine	0.59 ± 0.17 (9)	1.64 ± 0.41 (9)	10.4 ± 2.1 (6)

The compounds were administered in a dose equimolar to that of adenosine and were injected by the same route. Labelled glucose was administered at 0 time at a dose of 20 μCi/kg of body weight. The experimental animals were sacrificed 2 hr after the administration of the substances. Other experimental conditions as in fig. 1. The figures represent mean values ± standard error. Numbers in parenthesis are the number of experimental animals used.

the consequence of the glucocorticoid-mediated impairment in the glucose utilization by the epididymal fat pad [5]. The ability of various nucleosides and adenosine derivatives to modify the glycogenesis and lipogenesis in the selected tissues was investigated (table 1). In the parameters studied, none of the compounds tested had more striking effects than adenosine. However, this does not mean that adenosine by itself was responsible for the described effects, since the nucleoside might be metabolized inside the cell, for example, by being phosphorylated by adenosine kinase to AMP, ADP and ATP [6]. Preliminary work in our laboratory indicates that animals treated with adenosine show higher levels of ATP in the liver cell; this could reflect a higher charge which would favor the biosynthetic processes or the production of storage compounds [7]. The fact that AMP administration (table 1) was not so effective in lipogenesis and glucogenesis is not contradictory to this idea, if we consider the permeability barrier of cells for the phosphorylated compounds. Guanosine and inosine show also the effects described for adenosine, although to a lesser degree (table 1); those nucleosides might also be converted to adenine nucleotides inside the cell previous to transformation to hypoxanthine [8–10].

During the course of this work, some data [11] have appeared in the literature indicating that 3′5′-cyclic AMP produces in isolated fat cells some effects we ascribed here to adenosine in adipose tissue; on the other hand, an elevation of 3′5′-AMP in the liver would have opposite effects to those obtained with adenosine [12], for these reasons we can neither support nor discard the potential conversion of adenosine into cyclic AMP.

It is difficult to accept the idea that the actions here ascribed to adenosine might be directly mediated by the liberation of a hormone. None of the known hormones can reproduce in temporal sequence and in magnitude all the reported effects of adenosine; a "permissive" effect of some hormone can not be ruled out.

The drastic effects of adenosine on glucose metabolism in the liver and epididymal fat pad described in this paper could make a very useful tool of this substance in the study of hepatic glycogen metabolism and in the biosynthesis of lipids in the adipose tissue.

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