INACTIVATION OF RAT LIVER GLYCOGEN SYNTHETASE BY 3':5'-CYCLIC NUCLEOTIDES

W. H. Glinsmann and E. P. Hern

Section on Physiological Controls

Laboratory of Biomedical Sciences

National Institute of Child Health and Human Development

Bethesda, Maryland 20014

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SUMMARY

The 3':5'-cyclic phosphate esters of adenosine, guanosine, inosine, cytidine and uridine enhance the conversion of glycogen synthetase a to b in the perfused rat liver and in liver homogenates. This supports the possibility that these nucleotides activate a kinase which phosphorylates glycogen synthetase.

INTRODUCTION

The role of A-3':5'-P in controlling cellular metabolism is being extensively explored (1). Recent studies show the existence of specific pathways for the formation and degradation of 3':5'-cyclic nucleotides other than A-3':5'-P (2,3,4) and of metabolic effects of these nucleotides in isolated systems (5,6). This raises the possibility that they may also be involved in metabolic regulation. At our present state of knowledge, the most likely candidate for such a role is G-3':5'-P. Endogenous urinary excretion of G-3':5'-P (7) is altered by hormones (8), and the presence of distinct guanyl cyclase (4) and G-3':5'-P-phosphodiesterase (3) systems has been recently demonstrated.

In a previous study we investigated the effects of G-3':5'-P in isolated rat liver and adrenal systems (6) and found administration of this nucleotide produced many of the same effects as A-3':5'-P. Both compounds stimulated hepatic tyrosine aminotransferase induction, glucose output, gluconeogenesis and glycogenolysis, and adrenal steroidogenesis. The enhanced hepatic

glycogenolysis that accompanied their administration was associated with rapid activation of phosphorylase and inactivation of the active form of glycogen synthetase. Because of the similarity of the responses obtained with G-3':5'-P and A-3':5'-P, the possibility was considered that G-3':5'-P was indirectly altering intracellular A-3':5'-P metabolism. This, however, could not be demonstrated. Rather, the similarity in dose effectiveness of the nucleotides and the failure to detect any rise in tissue levels of A-3':5'-P following G-3':5'-P administration supported the idea that G-3':5'-P may act directly.

In the present study, we sought to obtain further evidence regarding direct effects of G-3':5'-P as well as to test for effects of other 3':5'-cyclic nucleotides. We focused on the behavior of liver glycogen synthetase (UDPG:glycogen α-4 glucosyltransferase) since the activity of this enzyme was markedly altered by G-3':5'-P administration in our previous study (6) and direct effects of nucleotides on the interconversion of enzyme forms are amenable to testing in a cell-free system (9).

MATERIALS AND METHODS

Normally fed male Osborne-Mendel rats (130-160g) were obtained from the NIH Pathogen-free colony on the morning of experimentation. A recycling liver perfusion apparatus was used as in previous studies on liver carbohydrate metabolism (6,10). A perfusate of washed sheep erythrocytes suspended (32% packed cell volume) in Krebs-Ringer bicarbonate buffer (pH 7.4) with 3% crystalline albumin and 20 mM glucose was circulated through the livers at 2.0 ml/g/min for 15 min. Portions of livers were then rapidly frozen between liquid nitrogen chilled aluminum blocks for future assay of glycogen synthetase activity.

Glycogen synthetase activity was measured at pH 8.0 as the rate of uniformly labeled ^{14}C-glucose (UDPG-^{14}C) incorporated into glycogen in the presence and absence of 5 mM glucose-6-P. The reaction mixture (150 μl) contained 50 μl of a 10% homogenate (30 mM Tris, 15 mM cysteine, 1 mM

EDTA, 20 mM NaF, and 400 mM sucrose) and 55 mM glycogen. UDPG concentrations were 3.3 mM and 10 mM (40,000 counts per minute (CPM)-^{14}C) in the absence and presence of glucose-6-P, respectively. Incubation for 15 min at 30°C was terminated by the addition of 1.0 ml 30% KOH, and glycogen was precipitated and washed twice with 67% ethanol. Aliquots of the glycogen suspended in water were then assayed for ^{14}C in a dioxane counting solution (75% efficiency).

Glycogen synthetase a kinase activity was determined in rat liver homogenates by the method of DeWulf and Hers (9). In this assay, liver homogenates, from which cell membranes containing adenyl cyclase were removed by initial centrifugation, were incubated with submaximally activating amounts of ATP and Mg^{++}, varying concentrations of nucleotides, and caffeine to inactivate cyclic nucleotide phosphodiesterases. After terminating the kinase activation by the addition of EDTA to remove Mg^{++}, a standard glycogen synthetase assay was performed omitting glucose-6-P. Activity of the glycogen synthetase a kinase then was estimated from the decrease in glycogen synthetase a activity (decrease in CPM ^{14}C-glucose incorporated into glycogen). Advantage was taken of the acute effects of an intravenous glucose injection (2g/Kg body wt) to convert glycogen synthetase to the active (a) form in vivo, prior to preparation of the liver homogenate (9).

All nucleotides were purchased from Boehringer-Mannheim with the exception of UDPG-^{14}C (213 mc/mmole) which was obtained from Tracerlab.

RESULTS AND DISCUSSION

Glycogen synthetase activity in liver, as in muscle, is thought to be controlled in part by a phosphorylation-dephosphorylation sequence (11,12). An active, dephosphorylated (a) form of the enzyme is converted to an inactive, phosphorylated (b) form by a kinase reaction requiring ATP and Mg^{++}. The b form can be activated by glucose-6-P (11,12). It has been proposed by Huijing and Larner that A-3':5'-P activates glycogen synthetase a kinase

by increasing the affinity of an allosteric site for Mg^{++} (13).

In a previous study (6), G-3':5'-P added to the perfusate recirculating through isolated livers from normally fed rats was found to be as effective as A-3':5'-P in acutely decreasing the activity of glycogen synthetase a, while neither nucleotide altered total (a + b) enzyme activity. Both cyclic nucleotides were maximally effective at a 0.5 mM concentration in this regard, although maximum activation of phosphorylase and maximal rates of hepatic glucose output and glycogenolysis were achieved at 1 mM nucleotide concentrations. In the present study (Table I) the addition of 1 mM I-3':5'-P, C-3':5'-P, or U-3':5'-P to the perfusate was also found to markedly lower glycogen synthetase a activity in perfused

Table I: Acute Effects of Nucleotides on Glycogen Synthetase Activity in the Perfused Rat Liver

Perfusate addition	Glycogen synthetase μ moles ^{14}C-glucose incorp. glycogen/g liver/hr.	
	a	a + b
None	26 ± 3.0	43 ± 3.0
A-3':5'-P	9 ± 2.2	45 ± 3.0
I-3':5'-P	10 ± 1.0	45 ± 3.0
G-3':5'-P	10 ± 1.0	46 ± 3.1
C-3':5'-P	10 ± 2.4	44 ± 3.0
U-3':5'-P	12 ± 1.0	40 ± 1.5
AMP	18 ± 2.5	44 ± 3.3
IMP	18 ± 2.4	45 ± 2.4
GMP	19 ± 3.0	40 ± 2.0
C-2':3'-P	23 ± 4.0	48 ± 3.4

All nucleotides were added to the perfusate at 1mM concentration and allowed to circulate for 15 min before rapid freezing of liver tissue. Four perfusions were performed in each group. Conditions for measuring active (a) and total (a + b) enzyme activity are described in Methods. Values = mean ± S.E.

rat livers. A questionable lowering of glycogen synthetase a activity was seen following AMP, IMP, and GMP administration, and no detectable response was obtained when C-2':3'-P was given. The effects of lower concentrations of nucleotides were not tested.

These observations were compatible with the possibility that all 3':5'-cyclic nucleotides may have a direct action on a kinase which converts glycogen synthetase a to b (11,12,13). However, it was also possible that cyclic nucleotides other than A-3':5'-P (and perhaps to a lesser extent the 5'-purine nucleotides) were primarily inhibiting A-3':5'-P catabolism by an action on cyclic purine phosphodiesterase (1,8). For this reason, we explored the effects of these nucleotides in a liver homogenate system of DeWulf and Hers (9) which assays the inactivation of glycogen synthetase a by a reaction which requires ATP and Mg^{++}. By analogy with the muscle enzyme which has been shown to be phosphorylated during this inactivation process (11), it is likely that a decrease in glycogen synthetase a activity is secondary to activation of a glycogen synthetase a kinase system (9).

From Figure 1, it can be seen that glycogen synthetase a can be

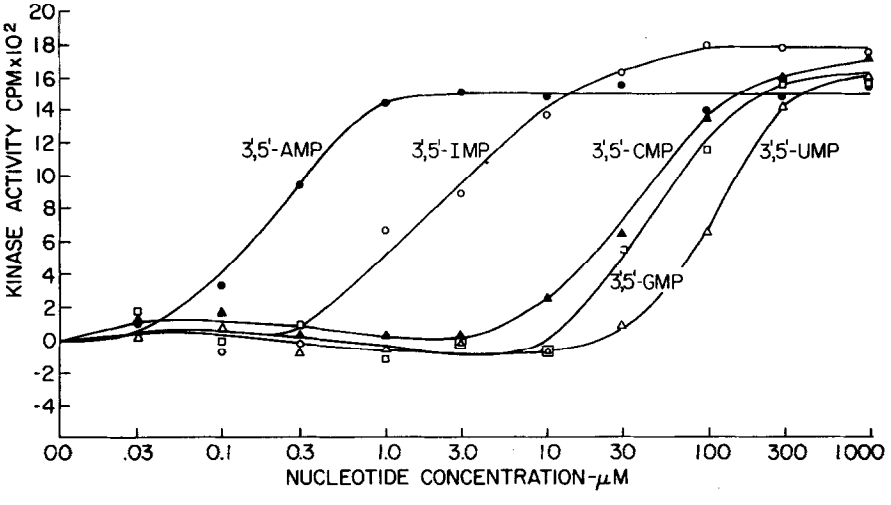

Fig. 1: 3':5'-cyclic nucleotide activation of glycogen synthetase a kinase in rat liver homogenate. Activity measurements represent observed decreases in glycogen synthetase a activity (CPM ^{14}C-glucose incorporation into glycogen) as described in methods.

inactivated to the same extent by all of the 3':5'-cyclic nucleotides tested, although on a molar basis, A-3':5'-P was 30 to 300 times more effective than the other 3':5'-cyclic nucleotides. That this is in fact due to a direct effect of these nucleotides on a Mg^{++} dependent kinase system is supported by the additional observations that none of these nucleotides lowered glycogen synthetase a activity when Mg^{++} was removed by EDTA, and that other nucleotide monophosphates (AMP, A-3'-P, GMP, IMP, CMP, UMP and C-2':3'-P), tested over the same concentration range, were without effect.

The results of the present study support the idea that 3':5'-cyclic nucleotides other than A-3':5'-P may have direct effects on the regulation of liver metabolism (6). However, there is little known about metabolic effects, tissue concentrations, and metabolic pathways involved in the metabolism of 3':5'-cyclic nucleotides other than A-3':5'-P. It is therefore difficult to speculate regarding the significance of the observed differences in nucleotide concentrations required to activate rat liver glycogen synthetase a kinase and the relative importance of each in regulating glucose and glycogen metabolism.

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