Studies on the Role of Adenosine 3′,5′-Monophosphate During Glucose Repression in Rat Liver (37538)

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Inhibition of *de novo* synthesis of enzymes by glucose and other carbohydrates is a common regulatory mechanism in bacteria. In mammals it has been shown that the *in vivo* and *in vitro* induction[1] of several hepatic enzymes by dietary or hormonal stimulation can be suppressed to a variable extent by the administration of glucose (1–3). Available evidence (4, 5) indicates that the induction of these enzymes is mediated by adenosine 3′,5′-monophosphate (3′,5′-AMP). It has been suggested that, as in bacteria, the addition of glucose to the internal environment may lower the intracellular levels of hepatic 3′,5′-AMP thus inhibiting the 3′,5′-AMP-dependent synthesis of enzymes (6–8).

In a previous report (9) it was demonstrated that intragastric administration of glucose inhibited the induction of hepatic serine dehydratase and tyrosine aminotransferase by glucagon but had no effect on the increase in 3′,5′-AMP in rat liver resulting from subcutaneous injection of the hormone. Since this finding indicated that glucose repression in mammalian liver may not be associated with changes in the levels of the cyclic nucleotide it was suggested, as one of the possible explanations, that glucose or its metabolite(s) may act indirectly by interfering with the mechanisms by which 3′,5′-AMP induces enzyme synthesis. Such an explanation would require no changes in the hepatic levels of 3′,5′-AMP during glucose repression.

In the present studies we have examined the possible interference of glucose with the initial steps of currently known pathways of 3′,5′-AMP action, including amino acid transport during glucagon stimulation, binding of 3′,5′-AMP to receptor proteins and 3′,5′-AMP activation of protein kinases in rat liver.

Materials and Methods. Young female Holzman rats were used for *in vivo* studies. In experiments designed to examine the effects of glucose on glucagon-stimulated uptake [3-^{14}C]α-aminoisobutyric acid by rat liver, all animals were housed for 17 days prior to hormonal treatment (at which time they weighed 140–150 g) in a windowless room with an automatic lighting switch which provided 12 hr periods of alternating darkness and light, the dark periods beginning at 8:00 AM. Food was made available to the rats immediately before the lights were turned off for an ensuing period of 8 hr. They were fasted the remaining 16 hr of each 24 hr cycle. Purina laboratory chow was fed for the first 12 days. Subsequently the animals were maintained on a pelleted protein-free diet for 5 days. No food was given on the day of the experiment.

The synthetic amino acid, [3-^{14}C]α-aminoisobutyric acid (New England Nuclear, sp act 5.3 mCi/mmole), was dissolved in 0.15 m*M* NaCl before use to make a concentration of 10 μCi/ml. Since this amino acid is slowly eliminated in the rat, it was given subcutaneously the day preceding the experiment in order to reduce the number of injections during its performance. At zero time, 2 mg glu-

[1] The term "enzyme induction" is used here to mean an acceleration in the rate of synthesis of a given enzyme. The mere increase in measurable activity does not imply that new enzyme is made available.

cagon/100 g of body weight (Eli Lilly and Co.) and 3 ml of a 70% glucose solution were given to rats as described previously (9). The rats were decapitated and fragments of livers were homogenized for 1 min with a Polytron PT-10 in 4 vol of Tris–HCl (pH 7.4) containing 0.1 m*M* dithiothreitol. One volume of 0.2 ml 100% cold trichloroacetic acid was added to 2 ml aliquots of the homogenate. After centrifugation (7000*g* for 10 min at 4°), 0.5 ml of the supernatant was added to 10 ml of scintisol and the radioactivity was determined in a Packard scintillation counter. Efficiency of counting was 90% and correction for quenching was made automatically, by external standardization. Samples of blood obtained after decapitation were processed in identical manner. The uptake of [3-^{14}C]α-aminoisobutyric acid by liver was estimated by the distribution of counts in blood and liver:

$$\text{distribution ratio} = \frac{\text{cpm/g liver}}{\text{cpm/ml blood}}.$$

The activities of serine dehydratase and tyrosine aminotransferase were determined (10, 11) in livers from the same animals, at various times during glucagon induction and glucose repression. Enzyme activity was expressed in micromoles of product formed per hour.

Rat liver fractions and partially purified 3′,5′-AMP binding protein preparations from rat liver were used to study glucose effects on the *in vitro* binding of 3′,5′-AMP to receptor proteins. The rats were sacrificed by decapitation and the livers were rapidly excised and chilled in freshly prepared, ice-cold 0.25 *M* sucrose in Tris–$MgCl_2$–KCl buffer (50 m*M* Tris–HCl (pH 7.5), 5 m*M* $MgCl_2$ and 25 m*M* KCl) as described by Blobel and Potter (12). The livers were blotted, weighed and minced in 9 vol of the same sucrose–Tris–$MgCl_2$–KCl solution. Homogenization was accomplished in a Potter-Elvejhem glass homogenizer fitted with a Teflon pestle and an ice-water jacket; 10–15 strokes at 1000 rpm were used for each sample. The homogenate was filtered through eight layers of gauze under slight vacuum. The filtrate was centrifuged at 2180*g* for 10 min at 0°. The postnuclear supernatant was removed and its volume was measured. The nuclear pellet (and all sediments from later steps) was resuspended by homogenization in the original volume of ice-cold sucrose–Tris–$MgCl_2$–KCl buffer. The postnuclear supernatant was centrifuged again under the same conditions. The combined mitochondrial pellets were taken up in the same volume of cold sucrose–Tris–$MgCl_2$–KCl as the nuclear fraction. The postmitochondrial supernatant was centrifuged at 78,480*g* for 90 min at 0°. The postmicrosomal supernatant was collected and the microsomal pellet was resuspended as indicated above. Aliquots from each fraction were removed during the procedure for 3′,5′-AMP binding and protein concentration determinations.

Purified beef muscle protein kinase was prepared essentially as described by Gilman (13). Peak II was utilized in this study.

Rat liver protein kinase was purified in a similar manner, except that DEAE-cellulose chromatography was performed by packing DEAE-cellulose equilibrated with 5 m*M* phosphate buffer (pH 7.0) in a special pasteur pipette, 8 mm in diameter. All buffers used contained 2 m*M* neutralized EDTA. The microcolumn was packed by gravity to a height of 4.5 cm. Small aliquots of the dialyzed ammonium sulfate fraction (1 ml, containing 18.5 mg protein) were chromatographed as needed. Elution was accomplished with 20 ml of a linear gradient of 5–300 m*M* phosphate buffer, pH 7.0. At least two peaks were resolved by this procedure; peak I eluted between 100 and 180 m*M* phosphate buffer while peak II, with the highest specific activity, eluted between 200 and 270 m*M* phosphate buffer. The entire chromatographic step took approximately 1 hr.

Binding activity was measured by a Millipore filter technique using [^{3}H]3′,5′-AMP in the presence of the protein kinase inhibitor (13). Glucose or other compounds were added to the incubation mixture at the desired concentrations, as indicated. Specific binding activity was defined as the number of picomoles of [^{3}H]3′,5′-AMP bound per milligram of protein, under the conditions of the assay.

Protein kinase activity was measured as de-

scribed before (14) in a 0.2 ml reaction mixture containing the following: 50 m*M* Tris–HCl buffer (pH 6.5), 5 m*M* $MgCl_2$, 225 μg/ml calf thymus histone (Sigma), 12.5 μM [γ-^{32}P]ATP (1×10^6 cpm/tube, New England Nuclear) and 25 μl of enzyme preparation (2–50 μg protein). Five micromolar 3′,5′-AMP, glucose or other compounds were added as indicated at the stated concentrations. The reaction was started by the consecutive and immediate addition of enzyme preparation and [γ-^{32}P]ATP. After 5 min incubation at 37° in a shaking bath, 0.1 ml of the mixture was pipetted onto filter papers (Whatman 3MM), 23 mm in diameter. The discs were placed immediately in a perforated plastic basket,[2] immersed in 100 ml of a cold solution of 10% trichloroacetic acid and 1% sodium pyrophosphate and the solution was agitated gently with a magnetic stirrer for 45 min. The basket was transferred to two successive 5% trichloroacetic acid solutions, also agitated by magnetic stirring, and the papers (still in the basket) were washed in ethanol–ether (50:50), and ether. They were then placed on a cardboard and dried under an infrared lamp. Protein-bound $^{32}P_i$ on the filters was counted in a scintillation mixture containing 5 ml of toluene with 0.4% Omnifluor (New England Nuclear). The combination of 1% pyrophosphate added to the trichloroacetic acid solution and the continuous agitation during washings, made possible by the use of the basket, insured reproducible low background radioactivity in the blanks. A unit of protein kinase activity was defined as the amount of enzyme which transferred 1 pmole of $^{32}P_i$ from [γ-^{32}P]ATP to recovered protein in 5 min at 37°, under standard assay conditions. Specific activity was expressed as units per milligram of protein. Protein concentration was determined by the method of Lowry *et al.* (15).

Results. α-Aminoisobutyric acid transport during glucose repression. As demonstrated in Fig. 1, control animals receiving diluent and saline showed a small decrease in the distribution ratio throughout the duration of the experiment. When glucagon was injected, after a slight decline at 20 min (which was similar to that of the controls), there was a sustained increase in the transport of α-aminoisobutyric acid into the liver. The activities of tyrosine aminotransferase and serine dehydratase were already elevated 2 hr after glucagon administration and increased sixfold and fourfold, respectively, after 4 hr (Fig. 2). The simultaneous administration of glucose did not substantially change the time-curve of distribution ratios obtained with glucagon alone (Fig. 1); in fact, higher distribution ratios were observed in rats given glucose plus glucagon at 2 hr. The hormonal induction of tyrosine aminotransferase was, however, repressed (25%) by glucose feeding and that of serine dehydratase by 35% (after correction for the control enzyme activities). The smaller degree of repression obtained compared with previous experiments (9) may be related to differences in weight and feeding schedules.

In vitro *studies of 3′,5′-AMP binding activity.* The 3′,5′-AMP binding affinity of crude cell fractions from normal rat liver, obtained as described in Methods, was examined in the presence and absence of 25 m*M* glucose. Table I shows that the highest specific activity is located in the postmicrosomal supernatant and that the addition of glucose has little influence, if any, on the 3′,5′-AMP binding capacity of the different fractions.

It may be argued that the presence of hypothetical inhibitor molecules in these crude fractions could mask a possible glucose interference with the binding reaction. That this is not the case was demonstrated with the use of purified rat liver preparations. Glucose, glucose-6-phosphate (G-6-P) or glucose-1-phosphate (G-1-P) were added at approximately physiological concentrations (5, 0.1 and 0.1 m*M*, respectively) and up to 10 times of those amounts, to incubation mixtures containing the dialyzed ammonium sulfate precipitate or DEAE-cellulose–Peak II fraction from rat liver. Table II shows that

[2] The basket was easily made in the laboratory by cutting out the top of a polyethylene bottle and punching numerous 5 mm holes in the body. After removal of the neck of the bottle, the inverted perforated top was used as a stand for the body, which served as the perforated basket. A magnetic bar could rotate freely under the stand.

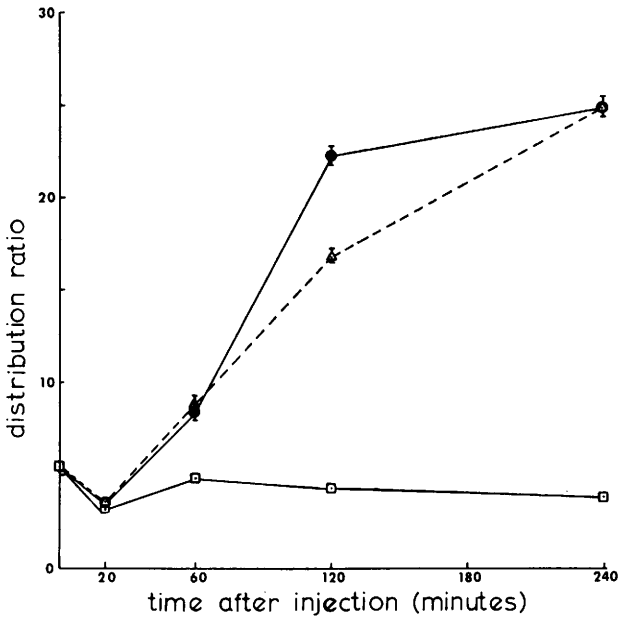

FIG. 1. The effect of glucagon and glucose on α-aminoisobutyric acid accumulation in liver. The methodology used is detailed in the text. Data was given as mean ± standard error of the mean (indicated by vertical bars) of three to five animals per point. The standard error falls within the character for those points where no standard error is indicated. (□—) diluent + saline; (△—) glucagon + saline; (●—) glucagon + glucose.

none of these compounds had any appreciable effect on 3′,5′-AMP binding.

In vitro *studies on protein kinase activity.* The possible interaction of glucose or glucose metabolite(s) with the catalytic unit(s) of protein kinases was examined. Since highly

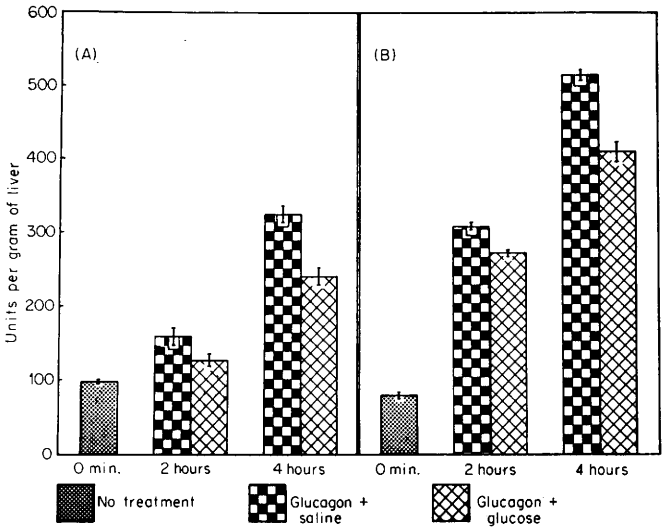

FIG. 2. The effect of glucagon and glucose on serine dehydratase (A) and tyrosine aminotransferase (B). Experimental details are given in the text. Mean values (columns) and standard error of the mean (vertical bars) of three to five animals per group are shown.

TABLE I. The Effect of 25 m*M* Glucose on 3′,5′-AMP Binding to Proteins from Normal Rat Liver.[a]

Crude cell fractions	Specific binding activity	
	— Glucose	+ Glucose
Homogenate	2.08	2.03
Nuclear suspension	0.86	0.80
Postnuclear supernatant	2.27	2.35
Mitochondrial suspension	1.37	1.31
Postmitochondrial supernatant	2.86	2.72
Microsomal suspension	1.24	1.76
Postmicrosomal supernatant	3.56	3.64

[a] Assays were performed in duplicate at pH 4.0, in the presence of protein kinase inhibitor. One picomole [^{3}H]3′,5′-AMP was added per tube. Glucose, when present, was at a final concentration of 25 m*M*. Values are the average of three experiments; the standard error for each point is within 4–10% of the mean.

purified protein kinases may not be representative of the entire set of protein kinases present in the liver cell, it seemed that studies using less pure preparations would involve all cellular protein kinases. Therefore, the effects of glucose, hexose and triose phosphates on the 3′,5′-AMP-dependent protein kinase activity of rat liver homogenate, postmicrosomal supernatant and partially purified rat liver protein kinase (ammonium sulfate precipitate) were explored.

Table III shows that in the presence of 3′,5′-AMP, none of the compounds tested, including fructose-6-phosphate (F-6-P), fructose-1,6-diphosphate (F-1,6-DP), 3-phosphoglycerate (3-PG), 2-phosphoglycerate (2-PG) and phosphoenolpyruvate (PEP), caused significant inhibition when added at approximately physiological concentrations, with the possible exception of 2,3-diphosphoglycerate, (2,3-DPG).[3] Only mild inhibition was obtained when their concentrations were increased to 10 times the normal levels (2-PG and F-1,6-DP, were assayed at 10 and 100 times the estimated physiological concentrations).

Comparison of the effects of any given compound on the various enzyme preparations (Table III) suggested that the low levels of inhibition became more noticeable as the purity of the preparation increased. Therefore, it was of interest to determine the results of adding some of those compounds to

[3] The concentration of 2,3-diphosphoglycerate in rat liver is not known with great accuracy. Data published by Grisolia and Joyce (16) indicate a level of >0.5 μmoles/g; a conservative estimate of the concentration derived from this figure is 0.1 m*M*.

TABLE II. The Effect of Carbohydrates on 3′,5′-AMP Binding to Receptor Proteins from Rat Liver.[a]

Carbohydrate	Concn (m*M*)	Dialyzed ammonium sulfate precipitate		DEAE-cellulose fractions (Peak II)	
		Sp act	% Inhibition[b]	Sp act	% Inhibition[b]
None	0	6.49	0	13.18	0
Glucose	5	6.67	(+) 2.8	13.21	0
	25	6.81	(+) 4.8	11.90	9
G-6-P	0.1	6.68	(+) 2.8	14.49	(+) 9.9
	1	6.57	(+) 1.3	13.58	(+) 3.0
G-1-P	0.1	6.71	(+) 3.4	13.29	(+) 1.0
	1	6.47	0	ND	ND

[a] Assays were performed in the presence of protein kinase inhibitor, at pH 4.0. [^{3}H]3′,5′-AMP (1 pmole/tube) was added. Total volume per tube = 70 μl. Results are the mean of three experiments; standard error for each point is within 5–10% of the mean.

[b] Values preceded by (+) denote percentage increase of activity over controls without carbohydrate. ND: not determined.

TABLE III. The Effect of Carbohydrates on Protein Kinase Activities from Rat Liver, in the Presence of 3′,5′-AMP.[a]

Carbohydrate	Concn (m*M*)	Homogenate	Postmicrosomal supernatant	Dialyzed ammonium sulfate precipitate
None	0	220.0[b]	702.4[c]	2434[d]
Glucose	5	227.0	713.5	2191
	25	245.0	698.1	1983
G-1-P	0.1	210.4	711.7	2225
	1	206.0	649.8	2120
G-6-P	0.1	203.8	708.4	2409
	1	215.3	680.3	2115
F-6-P	0.1	ND[e]	ND	2256
	1	ND	ND	2181
F-1,6-DP	0.1	ND	ND	2251
	1	ND	ND	1824
2,3-DPG	0.1	209.4	665.8	1921
	1	157.6	497.2	1531
3-PG	0.1	ND	ND	2175
	1	ND	ND	1902
2-PG	0.1	ND	ND	2070
	1	ND	ND	1951
PEP	0.1	220.2	ND	2269
	1	182.1	ND	1823

[a] Reaction mixture contained 12.5 μM ATP and 5 μM 3′,5′-AMP. Results are expressed as protein kinase specific activity. Each value is the mean of four separate experiments; standard error for each point is within 10–15% of the mean. See text for details of experiments.

[b] Specific activity in the absence of 3′,5′-AMP = 37.1.

[c] Specific activity in the absence of 3′,5′-AMP = 115.1.

[d] Specific activity in the absence of 3′,5′-AMP = 539.5.

[e] ND, not determined.

the purified protein kinases from rat liver. In either case, no significant interference with protein kinase activity was observed with glucose, 2-D-deoxyglucose, *N*-acetylglucosamine or pyruvate. G-1-P, G-6-P and F-6-P had some inhibitory action, which at concentrations of up to 5 m*M* was either smaller or similar to that found in less pure preparations. Only at very high concentrations (10–25 m*M*) was an inhibition of 20 to 38% seen. A consistent inhibitory pattern with increasing purification was, therefore, not present. Residual ammonium ion in the dialyzed ammonium sulfate precipitate may have enhanced the slight inhibitory action of the substances studied.

Because 2,3-diphosphoglyceric acid appeared to be the most effective inhibitor, it was tested over a wide range of concentrations for its effect on both the 3′,5′-AMP-dependent and 3′,5′-AMP-independent protein kinases from rat liver and from beef muscle.[4] The effects were similar with either enzyme (Table IV). The lower, physiological concentrations did not interfere with protein kinase activities in the presence or in the absence of 3′,5′-AMP. At concentrations 100 times higher, over 80% inhibition was achieved. The magnitude of the effect was similar on both

[4] Available evidence indicates (17) that protein kinases from various tissues and species differ in their regulatory units but that their catalytic component is identical. Beef muscle protein kinase having a high specific activity was used only as a complement to similar experiments on rat liver protein kinase.

TABLE IV. The Effect of 2,3,-Diphosphoglyceric Acid on 3′,5′-AMP-Dependent and 3′,5′-AMP-Independent Protein Kinases.[a]

2,3,-DPG conen (m*M*)		Protein-kinase[b]	
		Beef muscle[c]	Rat liver[d]
0.1	—3′,5′-AMP	ND	ND
	+3′,5′-AMP	7.3	(+) 8.4
0.5	—3′,5′-AMP	ND	ND
	+3′,5′-AMP	19.6	ND
1.0	—3′,5′-AMP	ND	ND
	+3′,5′-AMP	25.7	28.8
2.5	—3′,5′-AMP	ND	ND
	+3′,5′-AMP	44.2	46.7
5.0	—3′,5′-AMP	71.9	69.6
	+3′,5′-AMP	62.5	58.5
10.0	—3′,5′-AMP	85.7	92.1
	+3′,5′-AMP	80.3	78.6
25.0	—3′,5′-AMP	100	100
	+3′,5′-AMP	100	100

[a] Conditions of the assay were the same as those on Table III. Results are expressed as percentage inhibition of controls, assayed in the absence of carbohydrates. Data are the mean of two to three experiments; standard error for each point is within 10–15% of the mean.

[b] Values preceded by (+) denote percentage increase of activity over controls. ND: not determined.

[c] Specific activities were: (+) 3′,5′-AMP: 73,250; (—) 3′,5′-AMP: 33,300.

[d] Specific activities were: (+) 3′,5′-AMP: 7490; (—) 3′,5′-AMP: 2870.

3′,5′-AMP-dependent and 3′,5′-AMP-independent enzymes.

Discussion. While there is strong evidence (8) that glucose inhibits the synthesis of certain enzymes in *E. coli* by reducing the intracellular level of 3′,5′-AMP, glucose repression in mammalian liver appears to operate independently of the amounts of cyclic nucleotide in the cell (9). There are, however, indications that glucose effects in rat liver may still be interrelated with 3′,5′-AMP metabolism. For example, dibutyryl 3′,5′-AMP reverses glucose repression of amino acid-induced serine dehydratase (18) and phosphoenolpyruvate carboxykinase (EC 4.11.1.32) (19).

As a possible explanation for this apparent contradiction it has been suggested (9) that glucose could interfere with the pathways of 3′,5′-AMP action leading to the synthesis of specific proteins. If this is the case, then the glucose effect should be examined at the various metabolic steps currently known to be influenced by the cyclic nucleotide. Although the exact manner in which 3′,5′-AMP acts during protein synthesis in mammals is not well known, Figure 3 indicates several such steps at which glucose could alter 3′,5′-AMP action, without changing the intracellular levels of the nucleotide.

Transport of amino acids across cell membranes provides potential sites for the regulation of the hepatic amino acid pool. A case can be made for the need of a suitable amino acid pool to ensure *de novo* formation of specific proteins after stimulation by certain hormones, such as glucagon. Evidence supporting the postulation that 3′,5′-AMP mediates the transport of amino acids after glucagon administration has been presented by several authors (20, 21). The effects of glucose on the glucagon-stimulated amino acid transport in liver had not been reported previously. The results shown in Figs. 1 and 2 demonstrate that glucose does not cause a depression in the transport of α-aminoisobutyric acid although administration of the carbohydrate inhibits induction of serine dehydratase and tyrosine aminotransferase. The divergent effects of glucose on these glucagon-stimulated, 3′,5′-AMP-mediated phenomena could represent a serious objection to a key role for the cyclic nucleotide during glucose repression in mammals. Since glucose does not interfere *in vivo* with one of the transport systems ("A" System) stimulated by glucagon, it would seem unlikely that a change in the amino acid pool could explain the suppression of enzyme synthesis. This is further supported by a previous study by Jost *et al.* (22) showing relatively small differences in the amino acid pools of a group of rats receiving a mixture of essential amino acids and another group to which both amino acids and glucose were given.

A second possible site of glucose interference is the binding of 3′,5′-AMP to its recep-

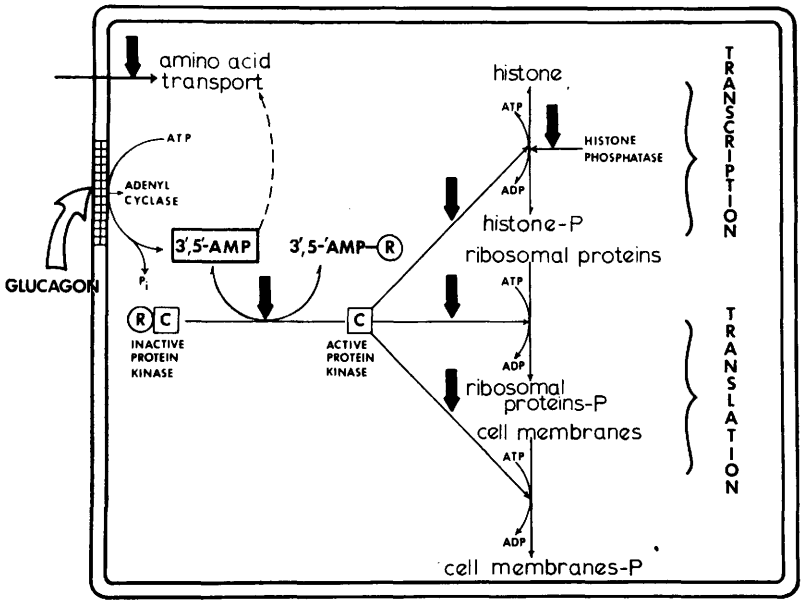

FIG. 3. Pathways of 3',5'-AMP action during synthesis of specific proteins in mammals. The nature of 3',5'-AMP mediation of stimulation of amino acid transport is not yet fully established; the broken line arrow merely indicates this effect. Thick inverted arrows denote possible points of glucose interference, without reference to stimulation or inhibition. (R) Regulatory units of protein kinase; (C) Catalytic unit of protein kinase.

tor proteins. Should glucose or a metabolite thereof interfere with binding of the nucleotide to the proteins through which its effects are manifested, changes in the glucagon-stimulated levels of 3',5'-AMP may not necessarily occur but induction of serine dehydratase and tyrosine aminotransferase could be repressed. The results presented here demonstrate the absence of inhibition of 3',5'-AMP binding by even higher than physiological levels of glucose or other carbohydrates.

The same end result as with receptor proteins would obtain if glucose or glucose metabolites inhibited 3',5'-AMP activation of protein kinases. Glucose and other carbohydrates studied in the present investigations had, in general, no effect on the protein kinase activities of several preparations examined. Only the triose and hexose phosphates caused some inhibition when tested at very high concentrations; 2,3-diphosphoglyceric acid was the most effective compound, inhibiting both the 3',5'-AMP-dependent and 3',5'-AMP-independent protein kinases equally. Since the amount of 2,3-diphosphoglyceric acid in liver is very small and since its levels during glucose repression are not known, the significance of this finding remains to be established. The slight inhibition due to other triose and hexose phosphates is probably nonspecific and not likely to play a regulatory role.

A few other possibilities would appear to remain from present knowledge for further studies of glucose interference with the mechanisms of 3',5'-AMP action. In particular, phosphorylation of ribosomal proteins and of cell membranes by protein kinases should be examined. Glucose effects at such levels may alter the stability of specific portions of the translational apparatus. Pitot and Jost (23) and Treadow and Khairallah (19) have presented evidence suggesting that glucose represses synthesis of serine dehydratase and phosphoenolpyruvate carboxykinase at the translational level. It is also conceivable that glucose may cause changes in the compartmentalized intracellular distribution of 3',5'-AMP. This would imply that without changing the total hepatic levels of 3',5'-

AMP, less cyclic nucleotide would be made available at the sites where it is necessary for the synthesis of specific enzymes.

The experiments described herein do not eliminate completely the possibility of an indirect effect by glucose or some of its metabolites; however, in view of our results an alternative mechanism (9) may deserve more serious consideration.

Summary. Several indirect mechanisms of glucose repression, not involving changes in the *in vivo* concentration of hepatic adenosine 3′,5′-monophosphate, were explored. Glucose, while repressing the induction of the serine dehydratase and tyrosine aminotransferase, does not interfere with the *in vivo* glucagon-stimulated, 3′,5′-AMP-mediated transport of α-aminoisobutyric acid in rat liver. No changes were noted in the *in vitro* binding of 3′,5′-AMP to receptor proteins in cell fractions and in purified preparations from rat liver when glucose or other carbohydrates were added to the reaction mixture. At physiological concentrations, glucose and other carbohydrates did not alter the phosphorylation of histones by hepatic or beef muscle protein kinases. At very high concentrations of several triose and hexose phosphates, only a slight inhibition of protein kinase activity was noted. Complete inhibition, however, was obtained with 25 m*M* 2,3-diphosphoglyceric acid.

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