CONTROL OF RAT LIVER GLYCOGEN SYNTHETASE AND PHOSPHORYLASE ACTIVITIES BY GLUCOSE

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Received April 16, 1970

SUMMARY

In the perfused rat liver, hyperglycemia causes a rapid inactivation of phosphorylase and a conversion of glycogen synthetase from a glucose-6-P dependent form to a glucose-6-P independent form. This effect is similar in the presence or in the absence of added insulin, but is absent following adrenalectomy and fasting when glycogen synthetase phosphatase activity is not detectable. Under conditions where phosphatase activity is low, glucose increases its activity. The effect of glucose occurs in the absence of detectable alterations in tissue concentration of adenosine 3',5'-monophosphate, the primary intracellular regulator mediating hormonal control of the activity of these enzymes. Circulating glucose levels also directly modify the effects of glucagon on the activities of these enzymes supporting the possibility that glucose levels may be important in moderating the control of liver glycogen metabolism in vivo.

Mammalian liver has an intrinsic ability to regulate circulating glucose concentration (1). Studies by Craig (2), Ruderman and Herrera (3), and Glinsmann, Hern, and Lynch (4) have clearly shown that hepatic autoregulation of circulating glucose concentration occurs in the absence of neural or hormonal intervention and that it is associated with changes in rates of gluconeogenesis and glycogen synthesis and degradation.

We were interested in this study in examining whether glucose-induced alterations in rates of glycogen synthesis and degradation were mediated by changes in the activities of the active (a) forms of glycogen synthetase

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(UDPglucose: α 1,4-glucan α 4-glucosyltransferase, EC 2.4.1.11) and phosphorylase (α 1,4-glucan:orthophosphate glucosyltransferase, EC 2.4.1.1). The activities of these enzymes are thought to be primarily responsible for regulating net glycogen turnover (5).

Studies by DeWulf and Hers (6) on the regulation of mouse liver glycogen synthetase activity in vivo have supported the idea that glucose may act directly (in the absence of circulating insulin alterations) to increase the conversion of the enzyme from an inactive to an active form. The observations of Bishop and Larner (7) and Bishop (8) using dog liver and of Gold (9) using rat liver have been interpreted to support the idea that insulin is primarily important in this regulation. The effects of glucose are thought to be permissive to an action of insulin. Our present findings that circulating glucose can directly and rapidly regulate the activities of glycogen synthetase-a and phosphorylase-a in an isolated perfused rat liver preparation agree with those in a recent report by Buschiazzo, Exton and Park (10). This in addition to the observation that insulin does not have this action, support the view of DeWulf and Hers.

That part of the action of glucose may be explained by an effect on a phosphatase system which diphosphorylates glycogen synthetase. This is suggested by the observations that the effect of glucose to convert glycogen synthetase b to a does not occur when the activity of this system is absent (adrenalectomy and fasting), and it acutely increases the activity of this system when its basal activity levels are low (adrenalectomy).

MATERIALS AND METHODS

The methodology involved in an isolated rat liver perfusion has been previously described (4). Bilateral adrenalectomies were performed 7 to 10 days before experimentation. Drinking water contained 0.45% saline and 25% glucose. Glucose was assayed by the method of Washko and Rice (11). Tissue levels of adenosine 3',5'-monophosphate were assayed by the isotope displacement method of Brooker, Thomas and Appleman (12) with modifications

as in a previous study (13).

For the assay of enzyme activities, tissue was frozen between liquid nitrogen-chilled aluminum blocks within 3 seconds of interruption of perfusate flow. Tissue was kept in liquid nitrogen until homogenization (glass-teflon tissue grinder -2 to $0^{o}C$) in 0.001M dithiothreitol (or 0.02M cysteine), 0.005M disodium EDTA, and 0.2M sodium fluoride. Glycogen synthetase activity was assayed as the rate of uniformly labeled ^{14}C-glucose UDPglucose-^{14}C) incorporated into glycogen in the presence and in the absence of 10mM glucose-6-P, as previously described (13). In the absence of glucose-6-P the a form of the enzyme is measured; in its presence, an inactive (b) form is activated and an estimate of total enzyme activity can be made (5). Phosphorylase activity was measured in the direction of glycogen synthesis from glucose-1-P in the presence of 0.001M 5'AMP (13). With liver phosphorylase, only the active form of the enzyme is assayable. Glycogen synthetase phosphatase was assayed by the method of Mersmann and Segal (14).

Crystalline glucagon and trypsin-treated zinc porcine insulin were donated by Dr. Walter Shaw of Eli Lilly Research Laboratories, Indianapolis, Indiana. Uniformly labeled UDPglucose-^{14}C (237mci/mmole) was obtained from New England Nuclear Corporation. Other reagents were purchased from California Biochemical Corporation.

RESULTS AND DISCUSSION

Alterations in circulating glucose concentration in the perfused rat liver rapidly alter the activities of the active forms of glycogen synthetase and phosphorylase (Table 1). Hyperglycemia increases glycogen synthetase-a activity and lowers phosphorylase-a activity; hypoglycemia produces reciprocal effects. The maximum response occurs 7 to 10 minutes after a change in circulating glucose concentration is made. This response decreases in magnitude after prolonged perfusion, but is still consistantly demonstrable after a 3 hour period of normoglycemic (130 to 160 mg/100ml

Table 1 Effects of Perfusate Glucose Concentration and Insulin on the Activities of Glycogen Synthetase and Phosphorylase in the Isolated Rat Liver

Perfusate Glucose Conc. (mg/100ml)	Insulin (μg)	Control Perfusion Time (min)	Exp. Time (min)	No.	Glycogen synthetase (μmoles ^{14}C glucose incorp. into glycogen/g/gr.)		Phosphorylase (μmoles Pi formed/g/min)
						+G-6-P	
0	0	0	15	8	5 ± 1.1	145 ± 6.9	11.5 ± 0.86
130	0	0	15	8	10 ± 2.1	154 ± 6.8	7.9 ± 0.16
130	1	0	15	4	11 ± 3.0	128 ± 5.7	
300	0	0	15	8	23 ± 4.1	142 ± 8.0	5.5 ± 0.26
500	0	0	15	8	55 ± 5.0	136 ± 7.4	3.2 ± 0.25
500	1	0	15	8	48 ± 6.1	152 ± 10.0	
500	4	0	15	4	44 ± 4.8	138 ± 8.2	
500	0	30	5	4	22 ± 5.0	148 ± 12.0	6.7 ± 0.18
500	0	30	10	4	37 ± 3.2	139 ± 10.1	2.2 ± 0.20
500	0	30	30	4	39 ± 4.2	150 ± 5.0	4.2 ± 0.28
130	0	120	10	4	8 ± 1.9	150 ± 12.1	
500	0	120	10	4	22 ± 3.8	128 ± 8.2	
160	0	180	10	4	6 ± 1.0	146 ± 9.3	
500	0	180	10	4	18 ± 2.0	138 ± 10.1	
500	1	180	10	4	22 ± 4.0	117 ± 11.1	
500	1	180	10	4	17 ± 3.4	130 ± 8.0	

Values represent means ± SE. In the initial series, glucose levels ± insulin was adjusted prior to perfusion and the experiment was terminated after 15 minutes. In the second series, a period of control perfusion at 130mg% glucose concentration preceeded the experimental period during which time perfusate glucose concentration was elevated to 500mg%; liver samples were taken at timed intervals thereafter. In the third series, control perfusion was also at 130-160mg% circulating glucose concentration. A 10 minute experimental period followed during which time glucose ± insulin was added to the perfusate.

glucose) control perfusion.

Insulin could be permissive in this system, but changes in circulating insulin levels are obviously not necessary to obtain an effect of glucose. The perfusate (washed sheep cell-Krebs-Ringer bicarbonate buffer) used has no detectable insulin; no endogenous means to alter circulating insulin concentrations are present in the isolated liver. When we tested for an effect of added insulin to augment the action of glucose (Table 1), none could be detected. Insulin in the perfused rat liver appears to inhibit glucose output and glycogenolysis only when these processes are stimulated by glucagon or a glucagon-like stimulus (15,16). To date we have not been able to demonstrate an acute effect of insulin alone to stimulate hepatic glucose uptake or glycogen synthesis in the isolated liver.

To further clarify the capacity of the glucose-effect on glycogen enzyme activity, we tested whether hyperglycemia was capable of modifying glucagon-induced alterations in the activities of the active forms of glycogen synthetase and phosphorylase. Hyperglycemia was markedly antagonistic to the effects of small doses of glucagon, but was not capable of modifying the effects of pharmacologic doses of the hormone (Table 2).

The effects of glucose to inhibit the action of glucagon raised the possibility that glucose may be acting to decrease tissue levels of A-3':5'-P, the intracellular mediator of glucagon effects (17). Such an action of glucose would be analagous to that observed in bacterial systems where glucose-inhibition of enzyme induction (catabolite repression) has been linked with its effect to promote a rapid efflux of A-3':5'-P from cells (18,19). We were, however, not able to show any effect of hyperglycemia to lower liver levels of A-3':5'-P in this study (Table 3). This agrees with the observations of Baschiazzo, Exton and Park (10).

From the present studies, it seems likely that mammalian liver rapidly and directly responds to changes in circulating glucose concentration by altering the interconversions of active and inactive forms of

Table 2 Effect of Glucagon and Glucose on Rat Liver Glycogen Synthetase and Phosphorylase Activities After 15 Minutes of Perfusion

Glucose conc. (mg/100ml)	Glucagon (μg)	Glycogen synthetase (μmoles ^{14}C-glucose incorp. into glycogen/g/hr.) -G-6-P	Glycogen synthetase +G-6-P	Phosphorylase (μmoles Pi formed/g/min.)
0	0.00	4 ± 1.20	148 ± 6.9	10.2 ± 0.90
0	0.06	3 ± 0.31	155 ± 12.8	24.0 ± 2.30
500	0.00	39 ± 7.20	146 ± 8.4	3.2 ± 0.26
500	0.06	28 ± 3.20	154 ± 15.5	8.3 ± 0.61
500	5.00	2 ± 0.20	160 ± 10.2	28.2 ± 2.40

Values represent means ± SE; 8 animals were used in each group. Perfusate glucose concentrations represent initial values at the start of the experimental period. Glucagon was added to the perfusate reservoir.

Table 3 Lack of Effect of Circulating Glucose Levels on Liver Concentrations of A-3':5'-P

Glucose conc. (mg/100ml)	A-3':5'-P conc. (nanomoles/g)
0	0.66 ± 0.02
130	0.89 ± 0.10
500	0.88 ± 0.05

Following 15 minutes of liver perfusion at different circulating glucose concentrations, the livers were rapidly frozen in liquid nitrogen. A-3':5'-P was assayed by an isotope displacement method (12). 4 livers/group; mean ± S.E. Maximal glucagon stimulation during the same time period gave values of 14.4 ± 1.64 nanomoles/g A-3':5'-P.

glycogen synthetase and phosphorylase. The pattern of enzyme activities support the possibility that increasing glucose concentration effects a net dephosphorylation of the enzymes (5,8,9,14,17). Holmes and Mansour (20) have further observed that glucose activates phosphorylase phosphatase. An action of glucose on phosphorylase and glycogen synthetase phosphatase activation would be consistent with the observed changes in enzyme activities.

In order to test this possibility, we examined whether the effect of

glucose could be obtained under conditions of adrenalectomy and fasting when glycogen synthetase phosphatase activation does not occur (14) (Figure 1). Under these conditions, the acute administration of glucose *in vivo* did not alter the activation of the phosphatase system (Figure 1), and no effect of glucose to alter glycogen synthetase-*a* activity could be demonstrated in the perfused liver (Table 4). Fasting alone did not diminish the glucose-induced enzyme response nor did adrenalectomy providing the animals were fed (Table 4). The effect of glucose may therefore have involved activation of the glycogen synthetase phosphatase system.

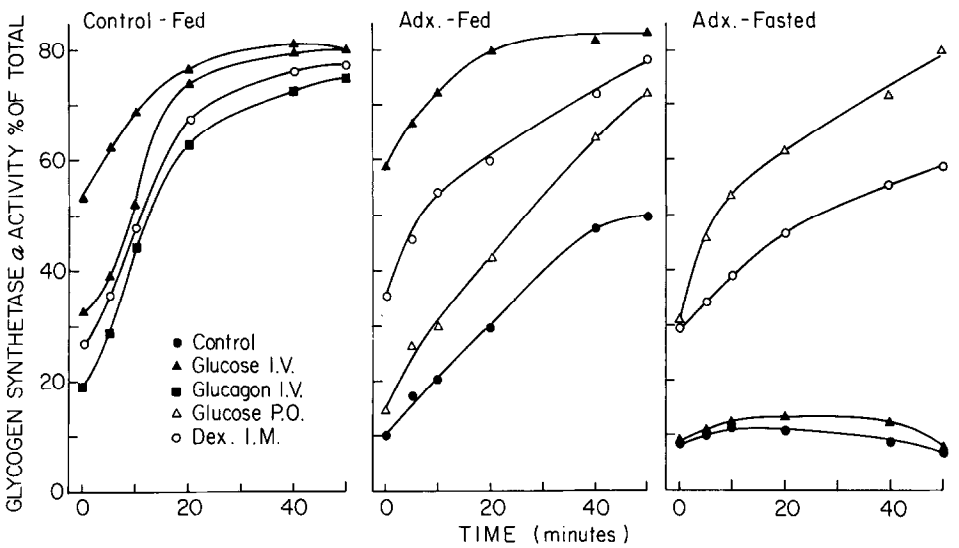

Figure 1. Glycogen synthetase phosphatase activation was assayed in the presence of Mg^{++} by the method of Mersmann and Segal (14). All experimental manipulations were done *in vivo*. The acute (15 min) effects of intravenous glucagon (5μg) or glucose (2g/kg) were compared to responses obtained after a 3 hour period following oral glucose (2g/kg at 0 and 90 minutes) or intramuscular dexamethasone (0.5mg at 0 minutes).

Further supporting this contention and also the idea that glucose was probably acting by a non-A-3':5'-P mechanism were the observations that the acute intravenous administration of glucose increased the glycogen synthetase phosphatase activation at times in the normal animal and routinely in the adrenalectomized-fed animal (where control rates were diminished) and

glucagon did not acutely affect this activation (Figure 1). Furthermore, the action of adrenal corticosteroids to increase the phosphatase activation could also be duplicated by the administration of oral glucose. In additional unpublished studies, this appears to be attributable to an action of insulin which requires continued protein synthesis. An action of insulin may be to maintain synthesis of the phosphatase or phosphatase activating system.

We currently are not able to explain fully the apparent discrepencies between our observations, which agree with those of DeWulf and Hers (6), and those of Bishop (7,8) and Gold (9) which support a primary requirement for insulin. Several points, however, should be considered regarding the action of insulin and glucose. It seems probable that the effects of insulin to increase the activity of a glycogen synthetase activating system takes several hours (9,14). This would not explain the acute effects of insulin *in vivo* to cause a conversion of glycogen synthetase-*b* to *a*. In several

Table 4 Effects of adrenalectomy and fasting on glucose induced changes in glycogen synthetase and phosphorylase activities in the isolated rat liver.

Glucose conc. (mg/100ml)	Exp.	Glycogen Synthetase (μmoles ^{14}C-glucose incorp. into glycogen/g hr.)	
			+G-6-P
130	normal-fed	14 ± 3.2	121 ± 10.4
500	normal-fed	45 ± 5.0	135 ± 4.6
130	normal-fasted	22 ± 3.0	115 ± 6.9
500	normal-fasted	68 ± 6.2	108 ± 12.4
130	adx.-fed	11 ± 3.0	121 ± 8.4
500	adx.-fed	50 ± 4.2	130 ± 8.0
130	adx.-fasted	5 ± 2.0	135 ± 10.2
500	adx.-fasted	7 ± 1.9	129 ± 12.0

Values represent mean ± S.E. A 15 minute experimental period was used following a 15 minute period of control perfusion with perfusate glucose concentration of 130 mg/100ml. Fasting was for 48 hours during which time adx. (adrenalectomized) animals received 0.45% saline for drinking water. 6 animals were used in each group.

experiments, Bishop (8) found that glucose alone given to pancreatectomized dogs caused a rapid activation of the glycogen synthetase phosphatase system. In other animals, insulin had an additional action on this system when it was given after glucose. In these experiments, higher circulating glucose levels were present when insulin was given, and the acute regulation of synthetase phosphatase attributed to insulin could then in part have been secondary to an effect of increased glucose concentration. In studies by both Bishop and Gold, the effect of insulin to cause a conversion of glycogen synthetase to an active form required concomitant hyperglycemia. In the experiments of Gold with insulin administration to 48 hour alloxen diabetic rats (9), an acute effect of insulin to increase glycogen synthetase-a activity before producing any effect on the synthetase activating system was detectable may have been secondary to an action of insulin to antagonize the effects of glucagon (15,16) and allow the effect of glucose to be expressed.

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