An Absolute Requirement for Insulin in the Control of Hepatic Glycogenesis by Glucose

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SUMMARY

Livers from normal, adrenalectomized, and diabetic rats were perfused in vitro in order to investigate the mode of action of insulin in the control of glycogenesis by glucose. Control of glycogen synthase and phosphorylase by glucose is completely lost in livers from 2 and 6 day alloxan diabetic rats. Three hour treatment of normal rats with anti-insulin serum results in a decrease in the effect of glucose on hepatic glycogenesis. Glucose infusion into isolated perfused livers from fed normal and adrenalectomized rats promotes an increase in glycogen synthase activation and phosphorylase inactivation. These data clearly demonstrate that the presence of insulin rather than glucocorticoids is an absolute requirement in the control of hepatic glycogen synthesis by glucose.

INTRODUCTION

There is experimental evidence, in vivo, that several hormones may be involved in glucose control of glycogenesis in liver. Kreutner and Goldberg (1) and DeWulf and Hers (2) studied the effects of glucose in the liver in vivo and found that glucose increased the conversion of glycogen synthase from the inactive (D) to the active (I) form. The effect of glucose was proven to be direct when Buschiazzo et al. (3) and Glinsmann et al. (4) demonstrated the same effect of glucose on glycogen synthase in the isolated perfused rat liver.

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Kreutner and Goldberg (1) demonstrated that hydrocortisone or glucose administered to alloxan diabetic rats had no effect on the activity of hepatic glycogen synthase, *in vivo*. The effectiveness of glucose could be restored by treatment of these animals with insulin. They also observed that activation of hepatic glycogen synthase by glucose proceeded normally in the fed adrenalectomized rat. Glinsmann *et al*. (4) demonstrated the lack of glucocorticoid involvement in glucose control of hepatic glycogen synthase in isolated perfused livers from fed adrenalectomized rats. In contrast, after 48 hour starvation of adrenalectomized rats, hepatic synthase activation was abolished (4,5). Gold (6) further reported that the synthase activating system (phosphatase) was lost in alloxan diabetic rats and was restored by insulin treatment.

Since we recently demonstrated a direct effect of insulin to activate glycogen synthase in the isolated perfused rat liver (7), the purpose of our present study was to determine the role of insulin in glucose control of hepatic glycogen synthesis using the same *in vitro* system.

MATERIALS AND METHODS

The technique of liver perfusion was modified from that of Mortimore (8) as described by Exton and Park (9). Livers from male Wistar rats were perfused at 37° with oxygenated (95% O_2-5% CO_2) Krebs-Henseleit bicarbonate buffer, pH 7.4, containing 3% Fraction V bovine serum albumin and 25% washed bovine red blood cells at a flow of 7 ml/min in a recirculating system. Bilateral adrenalectomies were performed 5 to 8 days prior to sacrifice. Diabetes was induced by intravenous injection of alloxan (60 mg/kg) and was diagnosed 2 days later by a maximum positive urine

sugar test. Guinea pig anti-insulin serum (AIS) was prepared by the method of Wright et al. (10). One ml of AIS (approximately 2 units) was injected intravenously 3 hours prior to liver perfusion, where indicated.

Aliquots of frozen liver powder were analyzed for glycogen (11) and glucose was determined by the alkaline ferricyanide method using the Technicon Auto-Analyzer. ^{14}C-glycogen was determined as previously described (12). Tissue glycogen synthase activity was assayed without added sulfate using the $\pm$ glucose-6-PO_4 filter paper assay of Thomas et al. (13). Tissue glycogen phosphorylase was extracted from frozen liver with 75 mM KF-3 EDTA-40 mM mercaptoethanol-20% glycerol at pH 6.1 (w:v, 1:10) and after centrifugation (8000 X g) at 2° for 10 minutes, the supernatant was diluted five fold with 50 mM MES-50 mM KF-60 mM mercaptoethanol at pH 6.1 and an aliquot assayed in the presence of 1.0 mM 5'AMP using the filter paper assay described by Gilboe et al. (14).

RESULTS

Table 1 shows the effect of 15 mg/min glucose infusion for the final 15 minutes into isolated perfused livers from fed normal, adrenalectomized, and 2 and 6 day alloxan diabetic rats. In livers from normal and adrenalectomized rats, glucose promotes a shift of glycogen synthase from the inactive (D) to the active (I) form along with a decrease in the activity of glycogen phosphorylase in agreement with Buschiazzo et al. (3) and Glinsmann et al. (4). These changes in synthase and phosphorylase activities resulted in 6 to 7 fold increases in ^{14}C-glucose incorporation into glycogen. Therefore, the effect of glucose to control hepatic glycogen synthesis does not appear to require the presence of adrenal steroids.

TABLE 1

Regulation of Hepatic Glycogenesis by Glucose in Perfused Livers from Fed Rats

Type	Glucose Infusion	Glycogen Synthase	Glycogen Phosphorylase	^{14}C-Glycogen
		%I	µmoles	µmoles
Normal	0	25 ± 2	54 ± 2	1.2 ± 0.1
"	+	45 ± 2[a]	44 ± 1[a]	5.2 ± 0.7[a]
Adrenex	0	16 ± 1	38 ± 2	0.9 ± 0.1
"	+	40 ± 3[a]	31 ± 2[c]	6.3 ± 0.9[a]
Alloxan Diabetic 2 day	0	8 ± 1	42 ± 6	0.1 ± 0.1
	+	8 ± 1[b]	41 ± 5[b]	0.4 ± 0.1[c]
Alloxan Diabetic 6 day	0	12 ± 1	39 ± 4	0.9 ± 0.2
	+	11 ± 2[b]	34 ± 3[b]	1.0 ± 0.2[b]

Livers were perfused with 60 ml of perfusion medium in a recirculating system for 45 min. After 30 min, one side was infused with ^{14}C-glucose and the experimental side infused with ^{14}C-glucose along with 15 mg/min cold glucose. Infusions were continued for 15 min. Synthase is expressed as % in the active form. Phosphorylase is expressed as µmoles glucose incorporated into glycogen per g liver per 10 min assay, and ^{14}C-glycogen is expressed as µmoles glucose incorporated into glycogen per g liver per hour. Perfusate ^{14}C-glucose specific activities for 0 and + glucose were the same. Data are expressed as means ± standard error of the means.

[a] p is less than 0.001 compared to proper control.

[b] Not significantly different from proper control.

[c] p is less than 0.01 compared to proper control.

When livers of either 2 day (acute) or 6 day (chronic) alloxan diabetic rats were perfused with the same concentrations of glucose, neither glycogen synthase nor glycogen phosphorylase was altered (Table 1). The increase in ^{14}C-glycogenesis seen

TABLE 2

Regulation of Hepatic Glycogenesis by Glucose in Perfused Livers from Fasted Rats

Type	Glucose Infusion	Glycogen Synthase	Glycogen Phosphorylase	Glycogen ^{14}C-glucose	Glycogen Total
		%I	µmoles	µmoles	µmoles
Normal	0	21 ± 2	48 ± 2	0.2 ± 0.1	1.2 ± 0.2
"	+	47 ± 5[a]	39 ± 2[a]	4.9 ± 0.7[a]	2.5 ± 0.2[a]
Adrenex	0	9 ± 1	28 ± 3	0.1 ± 0.1	0.2 ± 0.1
"	+	16 ± 2[b]	23 ± 1[c]	1.0 ± 0.2[a]	0.5 ± 0.1[d]
Normal AIS 3 hour	0	18 ± 1	48 ± 1	0.2 ± 0.1	1.9 ± 0.5
	+	38 ± 2[a]	46 ± 2[c]	2.8 ± 0.2[a]	3.2 ± 0.9[c]
Alloxan Diabetic 2 day	0	10 ± 2	41 ± 3	0.2 ± 0.1	27 ± 14
	+	14 ± 2[c]	42 ± 2[c]	1.0 ± 0.1[a]	48 ± 18[c]
Alloxan Diabetic 6 day	0	15 ± 5	31 ± 4	0.2 ± 0.1	8.8 ± 3
	+	18 ± 7[c]	30 ± 2[c]	1.3 ± 0.3[a]	7.6 ± 3[c]

Experimental procedures and expression of results are the same as described for Table 1. Total glycogen is expressed as µmoles of glucose per g liver.

[a] p is less than 0.01 compared to proper control.

[b] p is less than 0.025 compared to proper control.

[c] Not significantly different from proper control.

[d] p is less than 0.05 compared to proper control.

in the acute diabetic can probably be explained by a push or mass action effect of glucose since basal incorporation was at such a low starting level. Similar effects probably due to the

mass action of glucose are observed in Table 2. In the chronic diabetic where basal glycogenic levels approached normal (Table 1), no increase in ^{14}C-glycogenesis was observed. These data clearly demonstrate an absolute requirement for insulin in the control of hepatic glycogenesis by glucose.

Glucose produced an increase in glycogen synthase and a decrease in phosphorylase activities in livers of fasted normal rats (Table 2). Again, the enzyme changes resulted in increased glycogen deposition expressed as either ^{14}C-glycogen or total glycogen content. In contrast to the fed state, 18 hour fasting in conjunction with adrenalectomy caused decreases in glucose-promoted alterations of synthase and phosphorylase activities. This agrees with reports on starved adrenalectomized rats, both *in vivo* (5) and *in vitro* (4). The nutritional state of 2 and 6 day alloxan diabetic rats appears to have no effect on glucose control of glycogen synthesis. Again, the increased ^{14}C-glycogenesis is most likely due to the increased substrate supply. Total glycogen levels in these livers remain unchanged.

Normal fasted rats were injected with AIS three hours prior to perfusion in order to determine if a short term insulin deficiency could affect glucose control of hepatic glycogenesis (Table 2). Although glucose promotes the activation of glycogen synthase in livers of these rats, the level of activation was lower than that observed in normal rats (47% in normal to 38% with AIS). Glucose has no effect on inactivation of phosphorylase in the same livers. These decreases in alteration of enzyme activities produced by AIS treatment resulted in one half the ^{14}C-glucose incorporation into glycogen that was observed in normal livers while total glycogen levels remained unchanged. Therefore, the impairment in glucose control of hepatic glyco-

gen synthesis seen in 2 and 6 day alloxan diabetes is demonstrable after only three hours of insulin deprivation.

DISCUSSION

We previously demonstrated that insulin directly promotes activation of glycogen synthase in the presence or absence of glucagon in the isolated perfused rat liver (7). In the present report, we demonstrate for the first time that glucose has no effect on glycogen synthase or phosphorylase interconversions in isolated perfused livers from diabetic rats. The effect of AIS to diminish the response of glycogenesis to glucose supports the idea that the impairment is due to an insulin deficiency rather than a side effect of alloxan. Further, it demonstrates that the enzyme system that is affected by insulin deficiency is probably turning over at a rapid rate.

In 1967, Kreutner and Goldberg (1) found that glucose did not activate hepatic glycogen synthase, in vivo, when injected into alloxan diabetic rats. Gold (6) demonstrated that the glycogen synthase activating system (phosphatase) was lost in the alloxan diabetic rat. Therefore, the loss of control of hepatic glycogen synthesis by glucose in diabetic rats is probably due to an absence of either total and/or active phosphatase(s), since the phosphatase(s) has been shown to be the enzyme effected by glucose (2,15,16).

Our results agree with those previously reported by Buschiazzo et al. (3) and Glinsmann et al. (4) that glucose directly promotes activation of glycogen synthase and inactivation of glycogen phosphorylase in livers from normal rats. Also, alterations in these same enzymes by glucose are not impaired in livers of fed adrenalectomized rats (4), while fasting or starvation of adrenalectomized rats does result in an impairment or loss in the control

of glycogen synthase (4,5). In addition, we have demonstrated for the first time that glucose control of phosphorylase is also impaired in livers from fasted adrenalectomized rats. The report of Kreutner and Goldberg (1) suggests that the effect of glucocorticoids on this system involves insulin secretion since they were able to show no effect of glucocorticoids on glucose stimulated synthase activation in adrenalectomized-diabetic rats. On the other hand, insulin completely restored the effect of glucose on glycogen synthase in adrenalectomized-diabetics. Therefore, it appears that glucocorticoids are not required for the control of hepatic glycogenesis by glucose.

These data are generally consistent with the current concept of a common phosphatase responsible for dephosphorylation of synthase and phosphorylase (17,18) although other interpretations are equally possible. In general, control of synthase and phosphorylase by glucose tends to disappear and reappear simultaneously suggesting that their phosphatases are affected in the same way by insulin or that there is one effect of insulin on a common phosphatase. The one finding not consistent with the common phosphatase concept was that with AIS the phosphorylase control by glucose was completely lost while the synthase control was only slightly decreased. This observation is not in keeping with the hypothesis of Stalmans et al. (19) that glycogen synthase activity is controlled by the activity of glycogen phosphorylase.

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