PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 158, 496-501 (1978)

Inhibitory Action of High Levels of Glucose on Glucagon Effects in the Isolated Perfused Rat Liver[1] (40233)

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The isolated, perfused rat liver has been used in our laboratory to study metabolic changes induced in the liver by alterations in the composition of the perfusing medium. It was shown (1) that addition of 210–260 mg% glucose to the blood perfusate obtained from fasted rats resulted in a reduction of ketone bodies and urea in the liver effluent. At the same time, glycogen was deposited in these livers. Opposite results were obtained by adding glucagon to the perfusate. Glucagon in the perfusate raised ketone bodies and urea in the effluent (2). The data presented in this paper were obtained from experiments in which glucose and glucagon were both added to the perfusate in an attempt to reduce the metabolic effect of glucagon on the fasted rat liver.

Materials and methods. The technique of liver perfusion, the preparation of the perfusate (whole "fasted" blood) and the subsequent chemical determinations have been described previously (1, 2). Livers were obtained from normal, fasted (18 hours) male rats, Wistar strain, of 350–400 g.b.w., with *ad libitum* access to regular Purina Chow up to the beginning of the starvation period.

In the control liver perfusion, 4 ml of fasted rat serum was added to a total volume of 50 ml of whole blood from fasted animals, after the control sample of the effluent had been taken. In the next series of perfusions, glucose (100 mg) was dissolved in 4 ml of serum and added to the perfusate after the control sample was obtained as above. In the third series of experiments, 1 μg of glucagon dissolved in 1 ml of serum was added to the perfusate after removal of the control sample, producing a glucagon concentration of 20 ng/ml. We found this supraphysiologic concentration of glucagon was needed to stimulate ketogenesis and ureogenesis.

In the fourth series, glucose (200 mg/100 ml of perfusate) was added to the perfusate as above and the liver was perfused with this medium for 3 min. Following this exposure of the liver to a high glucose concentration, glucagon was added to the perfusate as in the third series described above. This procedure was followed because preliminary experiments had shown that 3 min exposure to the glucose enriched medium was the minimum time required to demonstrate a significant effect on the action of glucagon. The combined glucose–glucagon medium was perfused for an additional 87 min.

Results. After a 30-min period of equilibration, the control "fasted" perfused liver added glucose to the effluent blood in amounts which raised the blood glucose levels by 24 mg/100 ml of perfusate over 87 minutes (Table I). The addition of glucose to the perfusate was followed by an initial rise in the glucose level in the effluent and then by a fall, with an overall decrease of 40 mg/100 ml at the end of 87 min. In the next series, addition of glucagon alone to the fasting blood perfusate resulted in a continuous rise in the effluent blood glucose to 107 mg/100 ml after 87 min. In the last series, addition of both glucose and glucagon resulted in a significantly smaller increase of blood glucose (33 mg in 87 min) in the effluent as compared to that seen with glucagon alone ($P < 0.001$).

Measurement of ketone body (Table II) concentration in the effluent in each series of experiments revealed that the addition of glucose to the perfusing medium significantly reduced the high ketone levels observed in the control effluent ($P < 0.001$). On the other hand, addition of glucagon alone to the perfusate significantly increased the ketone body release into the liver effluent ($P < 0.0005$), while the simultaneous addition of both glucose and glucagon resulted in about the same amount of ketone bodies produced with glucose alone.

[1] Supported in part by USPHS No. AM16342-03 & Washington Heart Association Grants.

0037-9727/78/1583-0496$01.00/0

TABLE I. BLOOD GLUCOSE CONCENTRATION (mg/100 ml) IN THE EFFLUENT BLOOD FROM "FASTED" PERFUSED LIVERS*

	Minutes						
Perfusion addition**	0	3	30	60	90	Δ3–90	Δ3–90
Serum							
Avr.	115	117	127	134	141	+24[d]	+10.5
±SE	6.1	5.9	4.1	3.2	5.5	5.3	2.3
Glucose							
Avr.	122	221	210	196	181	−40[a]	−17.5
±SE	5.3	4.5	5.6	4.0	4.3	5.5	2.4
Glucagon							
Avr.	125	129	165	205	236	+107[a]	+46.9
±SE	6.0	6.1	6.4	8.2	5.9	8.0	3.5
Glucose plus glucagon							
Avr.	118	227	239	247	260	+33[a]	+14.4
±SE	4.7	5.1	3.8	6.7	3.7	6.5	2.8

* Fifty milliliters of whole blood, obtained from fasted rats, was used to perfuse livers weighing 12.7 ± 1.8 g removed from other fasted rats (323 ± 2.6 gbw).

[b] Each value represents the average of six perfusions with its SE.

[c] Values expressed as μmoles of glucose per gram of liver during 87 min (μmoles/g liver/87 min).

[d] Statistically significant ($P < 0.001$) when compared with any of the other groups.

Triglyceride levels in this system followed a different pattern (Table II). The control period was accompanied by a small increase in blood triglyceride levels (7 mg/100 ml of perfusate). Neither glucose alone, nor glucose plus glucagon when added to the perfusate, altered the liver output of triglycerides (Table II). The addition of glucagon alone suppressed the increase over the 90 minute period, shown in the other groups ($P < 0.001$).

In the case of blood cholesterol, the addition of glucose or glucose plus glucagon, had no observable effects on the net release of cholesterol from the perfused liver (Table II). The addition of glucagon alone, however, produced a significant reduction of the total cholesterol levels when compared with all other groups ($P < 0.001$).

Blood urea concentrations increased during the 90 minute control perfusion period (Table III). Addition of glucose alone or glucose plus glucagon significantly reduced the urea production by the liver ($P < 0.001$), while the addition of glucagon alone increased urea production.

Free fatty acid (FFA) levels in the effluent did not change significantly during the control period (Table III). Glucose, glucagon, or glucagon plus glucose significantly decreased the FFA level of the perfusate ($P < 0.001$).

Measurement of liver glycogen content (Table IV) revealed that perfusion for 90 minutes with the fasted rat blood did not alter this parameter significantly. Addition of glucose to the medium, however, increased the final glycogen content of the liver ($P < 0.001$) while glucagon addition without added glucose reduced the concentration of liver glycogen ($P < 0.001$). Glucose added with the glucagon significantly reduced the glucagon mediated glycogenolysis; but there was nevertheless a significantly lower glycogen level that was observed when glucose alone had been added to the perfusate ($P < 0.001$).

Liver triglyceride content was decreased by the end of all the experiments described above (Table IV). The addition of glucagon alone to the medium produced a significant fall in liver triglycerides ($P < 0.001$). Following the 90-min perfusion, liver cholesterol declined significantly (Table IV) in all groups except in the group receiving glucagon. In the latter case, liver cholesterol rose significantly.

Discussion. The central role of the liver in the regulation of systemic blood glucose is well established (3, 4). Isolated livers obtained from fasted rats have a low glycogen content and release smaller amounts of glucose into the effluent (2). The addition of glucose to the perfusate delivered to fasted livers results in the uptake of glucose by the livers (1). In our studies, we found that when glucose and glucagon were added to the perfusion medium, the glucose reduced the usual glucagon-mediated release of glucose by the liver into the effluent.

The cellular mechanism for such an interaction of glucose and glucagon at the site of

TABLE II. BLOOD KETONE BODIES, PLASMA TRIGLYCERIDES AND TOTAL CHOLESTEROL CONCENTRATION (mg/100 ml) IN THE EFFLUENT BLOOD FROM "FASTED" PERFUSED RAT LIVERS

Perfusion addition*	Minutes 0	30	60	90	Δ0–90	Δ0–90 (m)
Ketone bodies						
Serum						
Avr.	17	23	29	36	+19 (a)	+14.5
±SE	2.1	1.9	2.3	1.8	2.2	1.7
Glucose						
Avr.	16	21	24	27	+11 (b)	+8.5
±SE	1.9	2.2	3.5	2.0	3.3	2.5
Glucagon						
Avr.	19	33	45	59	+40 (c)	+30.6
±SE	3.2	3.6	4.1	3.9	4.1	3.1
Glucose plus glucagon						
Avr.	18	20	25	28	+10 (d)	+7.6
±SE	2.4	1.8	2.9	1.7	2.7	2.1
Plasma triglycerides						
Serum						
Avr.	29	32	35	36	+7 (e)	+0.62
±SE	2.5	1.2	1.9	1.3	1.9	0.17
Glucose						
Avr.	27	33	36	37	+10 (f)	+0.89
±SE	1.8	2.3	1.3	1.4	1.7	0.15
Glucagon						
Avr.	28	32	30	30	+2 (g)	+0.18
±SE	2.6	1.3	2.2	1.5	1.3	0.11
Glucose plus glucagon						
Avr.	28	31	34	37	+9 (h)	+0.80
±SE	3.1	1.3	2.2	1.5	2.2	0.19
Total cholesterol						
Serum						
Avr.	52	54	59	64	+12 (i)	+2.4
±SE	2.6	2.5	2.7	3.0	0.9	0.18
Glucose						
Avr.	49	52	57	62	+13 (j)	+2.6
±SE	3.1	2.6	2.2	2.8	1.0	0.20
Glucagon						
Avr.	54	49	46	41	−13 (k)	−2.6
±SE	2.6	2.0	2.5	3.0	1.2	0.24
Glucose plus glucagon						
Avr.	52	55	60	65	+13 (l)	+2.6
±SE	2.8	2.2	2.5	2.1	1.3	0.26

* Each value represents the average of 6 perfusions with its SE. (b) Statistically significant v. (a) and (c); $P < 0.001$. (c) Statistically significant v. (a), (b) and (d); $P < 0.001$. (d) Statistically significant v. (a) and (c); $P < 0.001$. (g) Statistically significant v. (e), (f) and (h); $P < 0.001$. (k) Statistically significant v. (i), (j) and (l); $P < 0.001$. (m) Values expressed as μol/g liver/90 min for ketone bodies, triglycerides and cholesterol.

the liver cell has not been clarified. In both dogs (5) and rhesus monkeys (6) it has been shown that changes in the level of glucose delivered to the liver have no significant effect on glucagon mediated increases in liver cAMP except in those experiments in which insulin is also added to the medium. In our experiments we did not add exogenous insulin to the perfusate. The perfusing blood was obtained from intact, fasted rats in which insulin may be presumed to be somewhat lower than in the blood of fed animals. We did not, however, measure insulin levels in our samples.

A possible explanation for the data we obtained in the combined glucose-glucagon infusions is suggested by the work of Buschiazzo *et al.* (7), which showed that phosphorylase stimulation by glucagon in perfused liver system could be inhibited by ad-

TABLE III. BLOOD UREA AND PLASMA FREE FATTY ACID CONCENTRATION IN THE EFFLUENT BLOOD FROM "FASTED" PERFUSED RAT LIVERS*

Perfusion addition*	Minutes 0	30	60	90	Δ0–90	Δ0–90 (g)
	Urea (mg%)					
Serum (4 ml)						
Avr.	20	24	29	34	+14 (a)	+18
±SE	2.2	1.2	1.7	1.5	1.0	1.3
Glucose						
Avr.	22	24	26	27	+5 (b)	+6.6
±SE	1.9	3.0	4.3	1.7	0.7	0.9
Glucagon						
Avr.	23	33	48	60	+37 (c)	+48.6
±SE	3.0	1.7	1.9	2.6	3.5	4.6
Glucose plus glucagon						
Avr.	20	23	25	26	+6 (d)	+7.9
±SE	2.5	1.3	2.2	1.9	0.6	0.8
	Free fatty acids (μEq/100 ml)					
Serum (4 ml)						
Avr.	0.41	0.40	0.38	0.40	−0.01 (e)	0.001
±SE	0.06	0.08	0.10	0.05	0.002	0.000
Glucose						
Avr.	0.38	0.32	0.28	0.23	−0.15 (f)	−0.012
±SE	0.03	0.07	0.05	0.04	0.02	0.002
Glucagon						
Avr.	0.42	0.30	0.23	0.20	−0.22 (f)	−0.017
±SE	0.04	0.07	0.06	0.04	0.04	0.003
Glucose plus glucagon						
Avr.	0.39	0.32	0.26	0.21	−0.18 (f)	−0.014
±SE	0.04	0.07	0.06	0.04	0.03	0.002

* Each value represents the average of six perfusion with its SE. (b) Statistically significant v. (a) $P < 0.005$ and (c) $P < 0.001$. (d) Statistically significant v. (a) $P < 0.005$ and (c) $P < 0.001$. (e) Statistically significant v. (f) $P < 0.001$. (g) Values expressed as μmol/g liver/90 min.
(b) vs (d) Not significant.

dition of glucose without any significant change in the usual increase in cAMP mediated by glucagon administration. We did not make cAMP determinations in our preparation, however.

No data exist as to the precise mechanism of the inhibition of ketogenesis by glucose in the hepatocyte during the administration of glucagon. It has been proposed that glucagon activates hepatic lipase via activation of the adenylate system (8, 9). This appears to be similar to the pathway of glucagon activation of the phosphorylase enzymes. The ketogenic effect of glucagon has been demonstrated in liver perfusion systems (7, 9, 10) as well as in liver slices (11) and homogenates (8); but there are at present no data to identify the site of action of glucose in the inhibition of hormone sensitive hepatic lipase.

The situation is complicated by the fact that fasting produces adaptive hormonal and enzymatic changes which do not respond immediately to the restoration of exogenous nutrients. For example, when glucose is added to the perfusate delivered to the liver from a well fed animal, there is a rapid and significant increase in the formation of triglycerides (10, 12, 13). When the liver is isolated from a previously fasted animal, however, the acute administration of glucose has no measurable effect on triglyceride production (7, 13, 14).

Thus, the intrahepatic utilization of FFA seems to vary with the level of FAA in the perfusate, and the flux of FFA from the plasma also varies with the previous nutritional state of the liver cells (13, 15, 16). Glucagon is lipolytic in the fasted liver (2) but these lipolytic effects are smaller than in fed livers. If glucose is added to the perfusate, subsequent addition of glucagon also elicits a smaller lipolytic effect by the hormone as compared to a nonglucose enriched preparation. Previous data show that glucose reduces

FFA oxidation in the liver while increasing hepatic triglyceride formation (1). Similarly, glucagon appears to inhibit cholesterol release by the liver (2, 10) and glucose counteracts this effect. The mechanism of this interrelationship remains to be clarified.

The sparing effect of glucose on liver protein catabolism has been well documented (7, 17, 18). Pestaña (19) showed that a high glucose diet fed *ad libitum* to rats decreased the activities of the proteolytic enzymes in the liver of these animals. The inhibition by glucose of glucagon-mediated liver ureogenesis which we observed may reflect a similar action of glucose-enriched perfusate on the glucagon mediated proteolysis.

The fasting state resembles the diabetic condition. In both cases enzymatic changes have been observed including an elevation of acetyl CoA amd fatty acyl CoA (20), with stimulation of oxidative pathways and inhibition of boisynthetic pathways. Glucagon plays a role in setting the rate of activity of these enzymatic systems. In the absence of insulin, there is evidence to suggest that the hepatocyte, like the alpha cell of the pancreas, is relatively insensitive to high blood glucose levels (21) with respect to restoration of normal function.

In the studies reported in this paper, we subjected a non-diabetic fasted liver to a contradictory set of metabolic signals as compared to the usual physiological condition of fasting. Normally, fasting results in a lowered blood glucose concentration *in situ.* Insulin levels are simultaneously depressed. In our perfused liver preparation we increased both the glucagon and the glucose levels in the perfusate and did not alter the low insulin level that is normally present in the blood of fasted animals. Under these conditions we observed that glucose added to the perfusate reduced the magnitude of the catabolic effects of glucagon on liver cells. These data may be interpreted to mean that a normal liver cell population responds appropriately to varying ratios of substrate-hormone in the medium such that elevations in available substrate inhibit endogenous substrate production usually induced by glucagon in the fasting state.

The diabetic liver, as observed in many other experiments, however, continues to produce endogenous substrate despite a high serum glucose concentration (1). Thus, it appears that adequate insulin is required to enable the hepatocyte to respond to the substrate signal but does not similarly affect the response to catabolic hormones.

Summary. Livers obtained from fasted rats were perfused with whole blood obtained from fasted rats and were found to release small amounts of glucose to the effluent. The addition of glucose to the perfusing medium resulted in glucose uptake by the liver. When glucagon was added to the perfusate, glucose was released into the effluent. In these studies the effects of a combined glucose and glucagon infusion into livers from fasted rats were tested. The catabolic effects of a supraphysiological concentration (6×10^{-9} *M*) of glucagon on liver metabolism of carbohydrates, fats and proteins were significantly rreduced when glucose was also added to the perfusate. Thes, a high glucose concentration in the perfusate overrides the usual effects of glucagon on "fasted" liver cells.

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Received September 1, 1977. P.S.E.B.M. 1978, Vol. 158.