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INHIBITION OF THE SUBSTRATE CYCLE GLUCOSE:GLUCOSE 6-PHOSPHATE BY PHYSIOLOGICAL CONCENTRATIONS OF FRUCTOSE IN PERFUSED RAT LIVER

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SUMMARY

Livers from fed rats were perfused with whole rat blood containing glucose labelled uniformly with ^{14}C and specifically with ^{3}H at position 2. Infusion of fructose at a concentration of 1.8 µmol/ml of blood significantly depressed perfusate glucose concentrations and increased the apparent utilization of both radioactive glucoses. The substrate cycle glucose:glucose 6-phosphate, as assessed by comparison of the apparent rates of utilization of [U-^{14}C] and [2-^{3}H] glucose and the actual rates, determined from glucose specific radioactivities during the perfusion, was almost completely inhibited by fructose.

INTRODUCTION

For the liver *in vivo*, the diet is the only significant source of fructose. Concentrations of 1.5-2.5 µmol of fructose/ml of blood are found in the hepatic portal vein of animals absorbing either the monosaccharide (1) or sucrose (2).

In perfused liver from fed rats fructose, at physiological concentrations, lowers blood glucose (3,4) and increases the uptake of infused glucose (4). We have recently observed that fructose, at these levels, markedly increased heat production in isolated hepatocytes from starved rats without a concomitant increase in O_2 uptake (5). As the release of $^{3}H_2O$ from [2-^{3}H] glucose was simultaneously enhanced, these changes were interpreted as increased substrate cycling at glucose:glucose 6-phosphate. In view of the proposed role of 'futile' substrate cycles in energy expenditure (6,7) and the glucose:glucose 6-phosphate cycle in particular in blood glucose regulation (6,7), it was decided to examine the effects of physiological levels of fructose on the metabolism of [U-^{14}C,2-^{3}H] glucose in perfused livers from fed rats.

METHODS

Livers from fed male Hooded Wistar rats (300-320 g) were perfused *in situ* for an experimental period of 60 min. The perfusate was 100 ml of whole rat blood dialyzed as described by Mayes & Felts (8) against a modified Krebs-

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Henseleit bicarbonate buffer (9) containing 10 mM glucose and mixed amino acids (500 mg/l) (8). The rate of perfusion was 1.2-1.4 ml/g of liver/min and the $\underline{P}O_2$ of the blood entering the liver was 90-100 mm Hg (12-13 kPa).

Fructose, as a solution in 0.15 M NaCl, was infused to maintain concentrations of 1.8 mol/ml (3,4). At the start of the infusion ('zero time') 5 μCi of [2-^{3}H] glucose and 2 μCi of [U-^{14}C] glucose (specific radioactivity 17.9 Ci/mmol and 336 mCi/mmol, respectively, both obtained from The Radiochemical Centre, Amersham, Bucks, U.K.) were added to the perfusate in 1 ml 0.15 M NaCl containing 10 μmol of glucose. Samples were taken at zero time and then at 15 min intervals (9). 1 ml of blood was deproteinized with 4 ml of absolute ethanol and portions of the supernatant were passed through ion-exchange columns. H_2O, glucose, lactate, pyruvate and amino acids were successively eluted and the radioactivity counted (9,10).

Blood glucose was determined enzymatically by a commercial preparation of glucose oxidase (Boehringer Mannheim Pty. Ltd., Mt. Waverley, Victoria 3149, Australia). Perfusate lactate and fructose were measured by the methods of Hohorst (11) and Bernt & Bergmeyer (12), respectively. Liver glycogen was determined as described previously (9).

There were 4 perfusions in each group and data are shown as the mean SEM. Statistical evaluation was by the analysis of variance and $\underline{P}<0.05$ was taken as the criterion of significance.

RESULTS AND DISCUSSION

Carbohydrate metabolism. Infusion of fructose resulted in a mean concentration of 1.77±0.17 μmol/ml of blood being maintained throughout the experiment. The mean total uptake of the ketose was 50.1±1.7 μmol/g of liver/h. No fructose was detected in blood from the control livers.

The concentrations of blood glucose and lactate and the rates of hepatic glucose output and lactate uptake are shown in Table 1. In control livers, blood glucose reached equilibrium within 30 min of zero time at 6.2 μmol/ml. This steady state is a balance between hepatic output and erythrocyte glycolysis. The former was calculated using a rate of glucose utilization of 1.9 μmol/ml of blood/h (3,4). Thus, in control livers there was a net uptake of glucose during the first 15 min of the perfusion and only during the final two sampling periods was there a net output. As observed previously in livers from fed rats (3,4), equilibrium blood glucose concentrations were significantly depressed below control levels by 1.8 mM fructose. During the period 0-30 min the fructose-infused livers removed significantly more glucose from the perfusate than controls but at equilibrium the net balance was unchanged.

Table 1. Concentrations of perfusate glucose and lactate and rates of hepatic glucose output and lactate uptake in perfused rat liver in the presence and absence of 1.8 mM fructose

Time	Glucose				Lactate			
	Concentration (μmol/ml of blood)		Output (μmol/g per h)		Concentration (μmol/ml of blood)		Uptake (μmol/g per h)	
	Control	Fructose	Control	Fructose	Control	Fructose	Control	Fructose
0	8.0 ±0.6	8.3 ±0.8	-	-	2.11 ±0.26	2.94 ±0.38	-	-
15	6.9 ±0.6	6.1 ±0.5	-14.1*** ±1.3	-42.4 ±5.8	-	-	-	-
30	6.3** ±0.7	4.5 ±0.4	-0.5*** ±1.5	-27.2 ±2.3	1.37** ±0.10	3.59 ±0.70	-34.4 ±3.6	-18.0 ±6.0
45	6.3** ±0.7	4.0 ±0.4	+8.0 ±5.2	-4.7 ±4.6	-	-	-	-
60	6.1** ±0.6	3.9 ±0.5	+8.2 ±3.2	+9.0 ±2.1	1.31** ±0.08	2.88 ±0.45	-24.5 ±1.4	-26.6 ±3.5

Livers from fed rats were perfused with 100 ml of whole rat blood at a flow rate of 1.2-1.4 ml/min per g of liver. Uptake of lactate and output of glucose were calculated as previously described (9). Negative values indicate uptake and positive values output. All data are shown as means±SEM for four animals. ** $P<0.01$, *** $P<0.001$, for control versus fructose-infused livers.

Perfusate lactate concentrations were significantly raised by fructose infusion (Table 1) due to elevation of the concentrations of glycolytic intermediates in the liver (3). However the mean uptake of lactate (Table 1) did not differ between the two groups so it is unlikely that the hypoglycaemic effect of fructose was due to inhibition of glucose synthesis. The concentrations of liver glycogen were similar in both groups with a mean value of 182±17 (4) in the controls and 195±16 (4) μmol of glycogen glucose/g of wet weight in livers perfused with 1.8 mM fructose.

Metabolism of [U-^{14}C,2-^{3}H] glucose. The apparent rates of utilization of ^{3}H and ^{14}C glucose are shown in Fig. 1. In control livers approximately 62 and 51% of initial radioactivity in [U-^{14}C] and [2-^{3}H] glucose, respectively, remained in the perfusate at the end of the experiment (Fig. 1a). The infusion of fructose significantly increased the apparent utilization of both radioactive glucoses so that only 34% of ^{14}C and 30% of ^{3}H initial radioactivity remained unmetabolized at 60 min (Fig. 1b).

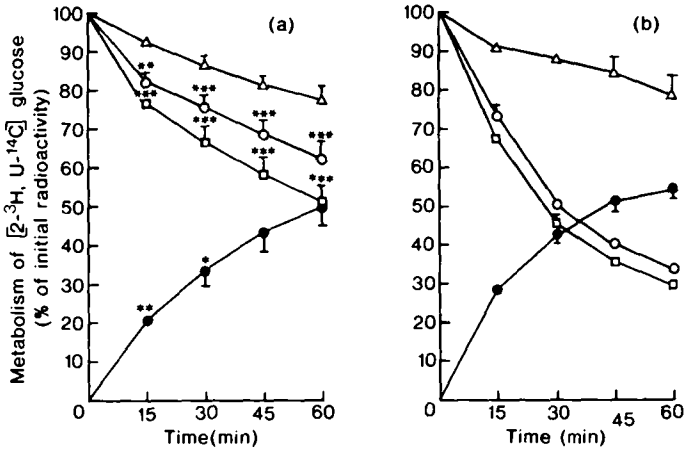

FIGURE 1. Metabolism of [2-^{3}H,U-^{14}C] glucose by perfused rat liver in the absence (a) and presence (b) of 1.8 mM fructose.

Livers from fed rats were perfused with 100 ml of whole rat blood containing 2 μCi of [U-^{14}C] glucose (○,n=4) and 15 μCi of [2-^{3}H] glucose (□,n=4). The ratio of ^{3}H/^{14}C in perfusate glucose (△,n=4) and $^{3}H_2O$ production (●,n=4) were determined as described in the text. Data are shown as means±SEM of four animals. * $P<0.05$, ** $P<0.01$, *** $P<0.001$ for control versus fructose-infused livers at the appropriate time interval.

With ^{14}C glucose, negligible quantities of isotope were recovered in lactate, pyruvate or amino acids. For ^{3}H glucose the sole products were $^{3}H_2O$ and ^{3}H glycogen. In control livers $^{3}H_2O$ formation (and [2-^{3}H] glucose uptake) exceeded [U-^{14}C] glucose catabolism by 30% (Fig. 1a), indicating an active substrate cycle at glucose:glucose 6-phosphate (13). In contrast, in the fructose-infused livers, during the period 15-60 min, the rate of disappearance of [2-^{3}H] glucose was not different from that of [U-^{14}C] glucose (Fig.1b). This indicates that the cycle was abolished after the first 15 min of the perfusion.

In order to accurately determine the effects of fructose on hepatic glucose metabolism corrections have to be applied for erythrocyte glycolysis (9). As fructose does not alter the rate of erythrocyte glucose catabolism, (1.9 μmol/h/ml of blood (3)), it is possible to determine the contribution of the red blood cells to labelled glucose metabolism during the course of the perfusions. Corrections to the isotopic data of Fig. 1a for erythrocyte glycolysis suggest that the net hepatic utilization of ^{14}C glucose was 17% and

that of ^{3}H glucose was 28%. In the fructose-infused livers the corresponding values were 46% and 50% of the initial radioactivity, respectively. The ratio of $^{3}H/^{14}C$ in perfusate glucose in the latter perfusions (corrected for erythrocyte glycolysis) remained constant at 0.93 during the final threequarters of the perfusions. These data further support the hypothesis that cycling at glucose:glucose 6-phosphate was inhibited by 1.8 mM fructose (10).

With the assumption that the sole products of erythrocyte metabolism of [U-^{14}C,2-^{3}H] glucose are ^{14}C lactate and $^{3}H_2O$ (9), a more accurate estimate of hepatic metabolism may be obtained from the mean specific radioactivity of perfusate glucose at each 15 min interval (9). In all livers the uptake of radioactive glucose and release of $^{3}H_2O$ were maximal at 15 min (Table 2). These rates then remained constant in controls for the remainder of the experiment with a significantly greater mean rate of $^{3}H_2O$ formation (and

Table 2. Metabolism of [2-^{3}H,U-^{14}C] glucose by perfused rat liver in the presence and absence of 1.8 mM fructose

Time	N	Control		Fructose	
		Total (μmol/h)	Liver (μmol/h)	Total (μmol/h)	Liver (μmol/h)
Apparent metabolism of [U-^{14}C] glucose					
0-15	4	671±80	482±80	946±92*	771±89*
15-30	4	283±3.2	113±4.3	765±95***	608±96***
30-45	4	227±29	60±29	424±18***	270±16***
45-60	4	241±48	83±49	325±57	178±55
Apparent metabolism of [2-^{3}H] glucose					
0-15	4	872±68	695±66	1144±104*	969±104*
15-30	4	402±7.6	232±10	805±94***	648±95***
30-45	4	356±46	189±46	477±60	322±59
45-60	4	335±58	178±57	394±57	247±57
Apparent $^{3}H_2O$ formation					
0-15	4	703±51	531±41	975±115*	788±104*
15-30	4	524±28	352±28	555±77	396±76
30-45	4	428±49	299±27	442±82	187±82
45-60	4	338±41	207±37	187±20***	41±21***

The experimental conditions were those of Table 1. Rates of ^{14}C and ^{3}H glucose disappearance and $^{3}H_2O$ production were obtained from glucose specific radioactivity calculated from the glucose concentration and radioactivity for each 15 min sampling period (9). Data are shown as means±SEM for four animals. * $P<0.05$, ** $P<0.01$, *** $P<0.001$ for control versus fructose-infused livers.

[2-^{3}H] glucose uptake) compared with ^{14}C glucose removal. In the fructose-infused livers rates of ^{14}C and ^{3}H glucose metabolism and $^{3}H_2O$ release declined from 15 min. No consistent difference was observed between the disappearance of ^{14}C glucose and ^{3}H glucose in the presence of 1.8 mM fructose after 15 min: a finding in agreement with the data of Fig. 1 which demonstrates that the hypoglycaemia due to fructose was accompanied by inhibition of the glucose: glucose 6-phosphate futile cycle.

One anomalous aspect of the results presented in Table 2 is that at 15 min in control livers and throughout the perfusions in fructose-infused livers, the formation of $^{3}H_2O$ was less than the uptake of ^{3}H glucose. This suggests that either there was incomplete equilibration at glucose 6-phosphate:fructose 6-phosphate (13) or that glucose 6-phosphate, after conversion to glucose 1-phosphate, was converted to glycogen. Both these possibilities are supported by the incorporation of ^{14}C and ^{3}H glucose into hepatic glycogen. The data obtained with [U-^{14}C] glucose indicated that 8.68±1.9 (4) μmol of glucose was incorporated into glycogen/h per g wet weight of liver in the controls and 11.85±2.41 (4) μmol/h per g of liver in the fructose-infused livers. The corresponding results for [2-^{3}H] glucose were 3.58±1.2 and 5.91±1.0 μmol/h per g respectively. While these results are not significantly different statistically the ratios of ^{3}H incorporation/^{14}C incorporation indicate that about 21% more ^{3}H was incorporated into glycogen in the presence of 1.8 mM fructose.

Although fructose increases glycogen deposition in liver when infused with glucose (4), there is no significant change in glycogen content when it is infused alone (3,4). Fructose can activate glycogen synthetase (14), but it would appear that, for glycogen to be laid down, a glucose load must also be supplied.

The present data confirm previous observations that in perfused livers from fed animals (3,4) as in vivo with animals of the same nutritional status (1), fructose at physiological concentrations is hypoglycaemic. These findings

are in complete contrast with similar studies using food-deprived rats and to investigations on the effects of fructose at high concentrations where hyperglycaemia ensues (14). In isolated hepatocytes from fasted rats, fructose at all concentrations stimulates glucose production and the glucose:glucose 6-phosphate cycle (5,13). However the present results demonstrate that the hypoglycaemia induced by physiological levels of fructose in livers from fed rats is accompanied by suppression of the glucose:glucose 6-phosphate substrate cycle.

To the best of our knowledge this is the first demonstration that an external agent can inhibit this cycle. It would be of great interest to determine whether other hypoglycaemic agents (e.g. insulin) produce similar direct effects on the glucose:glucose 6-phosphate cycle in the perfused liver and if these changes also occur *in vivo*.

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