SPECIFICITY OF THE GLUCOSE EFFECT ON HEPATIC GLYCOGEN SYNTHESIS: A GLUCOSE RECEPTOR IN LIVER

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1. Introduction

Glucose can control the synthesis and breakdown of glycogen in the liver, independently of its role as carbon source for synthesized glycogen. The evidence for this concept is of two main types. First, glucose can inhibit the activity of glycogen phosphorylase *a* in liver, and can thereby promote the activity of glycogen synthetase *a* [1–6], these being the rate-controlling enzymes in glycogen metabolism [1,2]. Secondly, glucose can stimulate the synthesis of glycogen in the liver [1,7]. This action is seen most clearly in the liver from starved animals, when glucose initiates the net synthesis of glycogen which derives its entire net carbon supply from gluconeogenic precursors [7].

This regulatory function of glucose in the liver has been characterised as being at least partly due to the effect of glucose on the phosphorylase *a* dephosphorylation process, which can initiate a sequence of events resulting in the activation of synthetase *a* phosphatase [1–3]. However, there is still some uncertainty regarding the controlling function of glucose in liver metabolism. Thus in diabetic animals, fructose can elevate the activity of synthetase *a* in the liver, but the associated net rate of glycogen accumulation is not as high as would be expected in the normal liver [8]. This defect in diabetes appears to involve the glucose-dependent control of synthesis of glycogen [8] but it cannot be the sequential activation system based on phosphorylase *a* phosphatase, which is defective, as this hypothesis predicts that high synthetase *a* activities (as were observed in response to fructose) should be associated with full net synthesis of glycogen [1–4]. Therefore this evidence from studies of diabetes suggests that a separate insulin-dependent glucose receptor may exist.

These considerations have prompted us to investigate further the glucose-dependence of hepatic glycogen synthesis. We have used the perfused liver preparation, in which rates of net glycogen synthesis equal to those observed in vivo can be obtained, in livers from starved [7] or fed [9] rats. In this paper, the effects of glucose analogues on net glycogen accumulation are described. Also, experiments with inhibitors of glucokinase and measurements of the dephosphorylation of phosphorylase *a* are described. The results reveal that galactose can promote net glycogen synthesis and support the notion that a hexose receptor exists in liver, other than that operated through the response of phosphorylase phosphatase.

2. Experimental

Livers of male (180 g) Sprague-Dawley rats starved from 10.00 h for 48 h were perfused with bicarbonate-buffered saline containing albumin and washed rat erythrocytes gassed with air/CO_2 [7,8]. When present, C_3-substrates were a mixture of lactate, glycerol and pyruvate (total concentration initially 10 mM, maintained by infusion of a 1 M solution (refs [8,10]).

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Glucose (30 mM) or a related hexose (also 30 mM) were also present. Net glycogen synthesis was followed in sequentially removed liver samples [7]. Glucose was measured by an enzymatic method essentially as described by Trinder [11] and glycogen was determined as glucose, after hydrolysis with fungal glucosidase [12].

Phosphorylase *a* phosphatase activity was assessed by the disappearance of endogenous hepatic phosphorylase *a* [3,13,14] during incubation at 20°C. Liver samples were homogenised in 4.5 vol. 50 mM glycylglycine, pH 7.0 and incubated at 20°C in the absence or presence of 30 mM glucose, mannose or galactose. After various time intervals samples were removed for assay of phosphorylase *a* [15] determined at 20°C by following the incorporation of ^{14}C from [U-^{14}C]glucose 1-phosphate (50 mM) into glycogen (1%, w/v) in the presence of 150 mM sodium fluoride, 10 mM EDTA and 0.5 mM caffeine, final pH 6.5. The rest of the procedure was as previously described [16].

Chemicals were the highest grade commercially available (for sources, see [8,10]). 2-Deoxy-D-glucose, D-mannoheptulose and D-mannose were obtained from Sigma Chemical Co. Ltd (London S.W.6, England) and the other compounds were from Koch-Light Laboratories Ltd (Colnbrook, Bucks, England). All hexoses or analogues were the D-form.

3. Results and discussion

In one group of perfusions, a variety of hexoses was included in the perfusion fluid, as replacement for glucose and the effect on the net accumulation of glycogen was assessed (table 1). Among naturally occurring hexoses, galactose (but not mannose) supported a substantial rate of synthesis. Although galactose can act as a precursor of glycogen when present in addition to glucose (table 1, see also ref. [17]) the high rate of synthesis obtained with galactose plus C_3-substrates (0.39 μmol of glycogen glucose/g/min, table 2) does not reflect a role of galactose as precursor (e.g., via UDP-glucose provision) as shown by the fact that the rate of synthesis with galactose alone was much lower (table 1). The glycogen synthesized in these perfusions was a glucose polymer, as our analytical procedure, based on the use of glucose oxidase, would not have detected any galactose in the glycogen.

Glucose was released into perfusate during all the present perfusions (tables 1 and 2) so that as in our previous studies [7–10], glycogen synthesis occurred without any net carbon provision from circulating glucose.

The role of substrates in glycogen synthesis in the liver from starved rats may therefore be summarized

Table 1
Carbohydrate metabolism in perfusions with naturally occuring hexoses

Experimental group	Hexose (mM)	Mixture of lactate, glycerol, pyruvate	Number of perfusions	Glycogen (μmol glucose/g)		Glucose (mM in perfusate)	
				20 min	50 min	20 min	50 min
1	Glucose (30)	+	3	4.6 ± 1.4	31.2 ± 0.2	30.3 ± 0.2	32.6 ± 0.5
2	Galactose (30)	+	4	3.0 ± 1.8	14.6 ± 5.6	1.8 ± 0.2	4.9 ± 0.3
3	Mannose (30)	+	3	2.5 ± 0.9	4.8 ± 3.5	2.0 ± 0.4	5.7 ± 0.3
4	Fructose (10)	+	3	9.8 ± 7.7	9.3 ± 4.4	3.3 ± 0.4	7.1 ± 1.0
5	Fructose (20)	+	4	6.9 ± 4.1	11.4 ± 4.6	3.4 ± 0.3	7.1 ± 0.4
6	Glucose (30)	–	5	29.8 ± 1.8	35.1 ± 8.8	31.6 ± 1.8	28.7 ± 1.7
7	Mannose (30)	–	3	1.7 ± 0.7	3.1 ± 1.1	2.9 ± 0.2	3.8 ± 0.1
8	Galactose (30)	–	3	2.8 ± 1.1	5.4 ± 2.5	2.8 ± 0.8	3.5 ± 0.7
9	Fructose (20)	–	3	7.2 ± 4.8	6.5 ± 5.8	2.7 ± 0.2	7.7 ± 1.0
10	Glucose (30)	–[a]	3	9.5 ± 0.7	25.0 ± 1.5	29.2 ± 0.5	30.7 ± 0.7

[a] Galactose (infused as a 0.5 M solution) instead of C_3-substrates

Livers were perfused with various substrates, as indicated. The mixture of lactate, glycerol and pyruvate, where present, was infused as 1 M solution. Further details are in the text. Results are means ± SE.

Table 2
Carbohydrate metabolism in perfusions with naturally occuring hexoses

Experimental group	Net glycogen synthesis (A)	Free glucose release (B)	Total glucose synthesized (A + B)
1	0.89 ± 0.17	0.93 ± 0.14	1.8 ± 0.24
2	0.39 ± 0.15	1.25 ± 0.12	1.64 ± 0.05
3	0.08 ± 0.12	1.44 ± 0.17	1.51 ± 0.20
4	−0.02 ± 0.21	1.53 ± 0.32	1.51 ± 0.35
5	0.15 ± 0.18	1.54 ± 0.29	1.69 ± 0.39
6	0.17 ± 0.09	−0.78 ± 0.15	0.61 ± 0.12
7	0.05 ± 0.04	0.37 ± 0.03	0.42 ± 0.06
8	0.09 ± 0.05	0.25 ± 0.06	0.28 ± 0.03
9	−0.02 ± 0.05	2.07 ± 0.38	2.05 ± 0.38
10	0.52 ± 0.11	0.56 ± 0.46	1.08 ± 0.56

For the experiments in table 1, rates of formation of glycogen (A) perfusate glucose (B) and total glucose (A + B) have been calculated (μmol glucose/g/min). Results are means ± SE.

as follows. If single substrates are present, or any combination that does not include glucose or galactose, rates of synthesis are low (< 0.2 μmol glucose/g/min). Further, the best single substrates are glucose (rate 0.17) and galactose (0.09) but rates in these conditions are very sub-maximal. High rates are only observed when glucose or galactose plus gluconeogenic substrates are present, and in the case of glucose at least, the hexose is then not consumed during synthesis.

The only simple theory which encompasses the above facts is that glucose and galactose can both act at a hexose receptor in the liver to promote glycogen synthesis (independently of their role as carbon-sources). To gain further insight into hexose action, some further glucose analogues of non-metabolisable type were tested in the perfused liver. The results obtained with mannose (tables 1 and 2) and some non-metabolisable glucose analogues (table 3) show that if glucose is slightly altered in the C_1, C_2, or C_6 position, the action to promote glycogen synthesis is diminished. The C_4-position (altered in the isomeric change between glucose and galactose) appears less critical for receptor binding. Also the configuration at C_2 in glucose is partially flexible, since some glycogen synthesis was observed with 2-deoxy-glucose substituted for glucose.

There is a possibility that galactose was initiating glycogen synthesis in the liver by producing glucose. However, this idea may be discounted on two grounds:

(i) The glucose concentration in perfusate in the perfusions with galactose plus C_3-precursors was only about 5 mM, which would not support synthesis at such a high rate in these conditions [7].

(ii) Mannose and fructose can generate glucose faster than galactose (and other products as rapidly as glucose, ref. [18]) but do not promote glycogen synthesis (table 1) although fructose in particular can serve as a (glucogenic) source of glycogen-carbon, in the presence of glucose. It is interesting that galactose can exert a stimulatory effect on glucose metabolism in adipose tissue [19].

Naturally occurring sugars were also tested further. Since the K_m of glucokinase for mannose is higher than that for glucose [20], the concentration of this sugar in perfusate was increased to 50 mM. Net glycogen synthesis was not improved (results not shown). Disaccharides, namely trehalose, sucrose and maltose were substituted for glucose, in the presence of the glucogenic mixture. Negligible rates of glycogen synthesis were obtained (results not shown).

The next question that arose was whether glucose and galactose promote glycogen synthesis in the liver as free hexoses or by conversion to a metabolite in the liver (e.g., UDP-hexoses). This question was tackled in the case of glucose action with the use of two inhibitors of the glucokinase reaction, namely, glucosamine [20] and mannoheptulose [21]. These agents did not markedly inhibit synthesis (table 3),

Table 3
Glycogen synthesis in the presence of analogues of glucose

Hexose (30 mM)	Other additions (5 mM)	Number of perfusions	Glycogen (μmol glucose/g)		Glucose (mM in perfusate)		Calculated net rate of glycogen synthesis (μmol/glucose/g/min)
			20 min	50 min	20 min	50 min	
Glucose	–	3	4.6 ± 1.4	31.2 ± 6.0	30.3 ± 0.2	32.6 ± 0.5	0.89 ± 0.17
2-Deoxy glucose	–	4	7.0 ± 5.9	13.4 ± 8.4	–	–	0.21 ± 0.09
6-Deoxy glucose	–	2	2.2	4.1	–	–	0.07
Methyl glucoside	–	4	18.1 ± 11.9	20.7 ± 10.4	2.0 ± 0.6	5.5 ± 0.8	0.09 ± 0.09
Glucose	Manno-heptulose	3	5.3 ± 0.8	23.2 ± 1.3	28.3 ± 0.9	30.0 ± 0.9	0.60 ± 0.06
Glucose	Glucosamine	3	3.6 ± 3.6	15.4 ± 4.9	28.6 ± 0.4	31.2 ± 0.8	0.39 ± 0.05

Livers were perfused with a mixture of lactate, glycerol and pyruvate infused as 1 M solution plus glucose and/or glucose analogues. Other details are in the text. Results are means ± SE (or of 2 measurements).

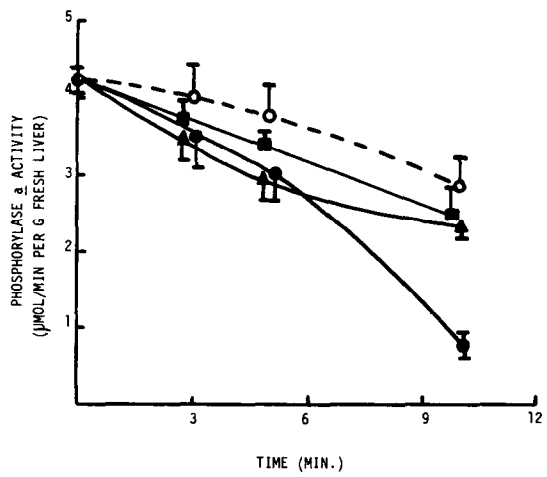

Fig.1. Decline in phosphorylase *a* activity in the presence of various hexoses. Homogenates from rapidly-frozen livers of fed rats were incubated at 20°C. After various times, an aliquot was added to a buffer containing fluoride and the activity of phosphorylase *a* was assayed (and calculated each time per gram of original wet liver). Various hexoses (final concentration 10 mM) were present during incubation of the homogenate. Glucose (●), galactose (■), mannose (▲). Control homogenates (○) contained no added hexose. Other details are in the text. Results are means ± SE of three measurements.

especially when they may have removed the contribution of glucose as carbon-source (0.17 μmol glucose/g/min) from the synthesis process.

Therefore, it appears that the free hexoses, glucose and galactose, themselves initiate glycogen synthesis in the liver.

Finally, we investigated the possibility that galactose could affect the phosphorylase *a* phosphatase receptor mechanism, as can glucose [1–3]. The glucose-effect on the phosphatase was confirmed (fig.1) whereas galactose and mannose exerted no stimulatory action (fig.1). Such a lack of phosphatase-response to mannose and galactose has been shown previously [21]. However, some inactivation of phosphorylase *a* was observed in later work with methyl glucopyranoside and 2-deoxy-glucose, the activity that remained being 40% and 50% of the initial [14]. Yet the present data show that neither of these glucose derivatives is capable of substituting for glucose in promoting glycogen synthesis, which may be explained by the fact that phosphorylase *a* activity has to be reduced to about 10% of the total phosphorylase in the liver [3], before deinhibition of synthetase phosphatase and hence glycogen synthesis can occur. Alternatively, our data with methyl glucoside and 2-deoxy glucose again suggest that the receptor operated by phosphorylase phosphatase is not the only hexose receptor in liver.

Thus the galactose effect to initiate glycogen synthesis is not exerted on the hexose receptor which is operated through phosphorylase *a* phosphatase [1–3]. This lends support (albeit indirect) to the possibility that glucose can also act through a second receptor, suggested previously from experiments with livers from diabetic rats [8], which exhibit a deficiency in glucose-dependent control of glycogen synthesis, as does the liver from fasted adrenalectomized rats [23]. In both states, the deficiency reflects insulin lack [8,23]. This inherent hepatic defect in carbohydrate metabolism is associated with an inability of the hepatic enzymes of glycogen metabolism to respond to glucose [8,23]. The evidence, that the defect is not in the glucose receptor operated through the phosphorylase phosphatase was derived from the effect of fructose, which could stimulate the activity of synthetase *a*, but full net synthesis capacity was nevertheless not restored [8]. Insulin did restore this capacity, after administration in vivo only, so this glucose receptor may be insulin-dependent.

In summary then, the present results provide a second line of evidence that a hexose receptor exists in liver, which serves to promote glycogen synthesis, may be insulin-dependent, and is not phosphorylase *a* phosphatase. This conclusion is reminiscent of those drawn regarding the existence of hexose receptors in the endocrine pancreas, where diabetic animals also have a defective β-cell glucose receptor [24]. The details of the pancreatic β-cell hexose receptor which promotes insulin secretion do not resemble those of the liver receptor, however. Thus galactose is not an effective insulin secretagogue, whereas mannose does stimulate secretion [25,26]. There may be a closer similarity between the particular hepatic hexose receptor revealed by the present work and the α-cell glucose receptor, suggested to be insulin-dependent in vivo but not respondent to insulin in vitro [27].

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References

[1] Hers, H. G., Stalmans, W., De Wulf, H., Laloux, M. and Hue, L. (1974) Alfred Benzon Symp. 6, 237.
[2] Hers, H. G. (1976) Ann. Rev. Biochem. 45, 167.
[3] Stalmans, W., De Wulf, H., Hue, L. and Hers, H. G. (1974) Europ. J. Biochem. 41, 127.
[4] Miller, T. B., Hazen, R. and Larner, J. (1973) Biochem. Biophys. Res. Commun. 53, 466.
[5] Hue, L., Bontemps, F. and Hers, H. G. (1975) Biochem. J. 152, 105.
[6] Glinsmann, W., Pauk, G. and Hern, E. (1970) Biochem. Biophys. Res. Commun. 39, 744.
[7] Hems, D. A., Whitton, P. D. and Taylor, E. A. (1972) Biochem. J. 129, 529.
[8] Whitton, P. D. and Hems, D. A. (1975) Biochem. J. 150, 153.
[9] Whitton, P. D. and Hems, D. A. (1975) Horm. Metab. Res. 7, 524.
[10] Hems, D. A. and Whitton, P. D. (1973) Biochem. J. 136, 705.
[11] Trindner, P. (1969) Ann. Clin. Biochem. 6, 24.
[12] Lee, E. Y. C. and Whelan, W. J. (1966) Arch. Biochem. Biophys. 116, 162.
[13] Stalmans, W., De Wulf, H., Lederer, B. and Hers, H. G. (1970) Europ. J. Biochem. 15, 9.
[14] Stalmans, W., Laloux, M. and Hers, H. G. (1974) Europ. J. Biochem 49, 415.
[15] Stalmans, W. and Hers, H. G. (1975) Europ. J. Biochem. 54, 341.
[16] Das, I. and Hems, D. A. (1974) Horm. Metab. Res. 6, 40.
[17] Sparks, J. W., Lynch, A. and Glinsmann, W. H. (1976) Metab. (Clin. Exp.) 25, 47.
[18] Wood, F. C., Jr, Leboeuf, B., Renold, A. E. and Cahill, G. F., Jr (1961) J. Biol. Chem. 236, 18.
[19] Naito, C. and Okada, K. (1974) J. Biol. Chem. 249, 1657.
[20] Parry, M. J. and Walker, D. G. (1966) Biochem. J. 99, 266.
[21] Hernandez, A. and Sols, A. (1963) Biochem. J. 86, 166.
[22] Stalmans, W., De Wulf, H., Lederer, B. and Hers, H. G. (1970) Europ. J. Biochem. 15, 9.
[23] Whitton, P. D. and Hems, D. A. (1976) Biochem. J. 156, 585.
[24] Robertson, R. P. and Porte, D., Jr. (1973) J. Clin. Invest. 52, 870.
[25] Lin, B. J. and Haist, R. E. (1975) Endocrinology 96, 1247.
[26] McDonald, M. J., Ball, D. H., Patel, T. N., Lauris, V. and Steinke, J. (1975) Biochim. Biophys. Acta 385, 188.
[27] Matchinsky, F. M., Pagliara, A. S., Hover, B. A., Pace, C. S., Ferrendelli, J. A. and Williams, A. (1976) J. Biol. Chem. 251, 6053.