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Effects of short-term insulin deficiency on lipogenesis and cholesterol synthesis in rat small intestine and liver *in vivo*

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The rate of lipogenesis in rat intestine increased on oral glucose loading and decreased after induction of acute insulin deficiency with streptozotocin. The latter effects could be partially reversed by administration of insulin. Parallel changes in the rate of lipogenesis were found in liver. In contrast, insulin deficiency did not alter the rate of cholesterol synthesis in intestine, but decreased it in liver. The physiological significance of the regulation of intestinal lipogenesis by insulin is discussed.

INTRODUCTION

Despite the existence of insulin receptors on rat intestinal cells (Bergeron *et al.*, 1980; Forgue-Lafitte *et al.*, 1980), no marked effects of insulin on the function or metabolism of small intestine *in vitro* have been demonstrated (Crane, 1961; Fromm *et al.*, 1969; Olsen & Rosenberg, 1970; Leese & Mansford, 1969, 1971). More recently, Kellett *et al.* (1984) have shown that acute insulin deficiency *in vivo*, induced by injection of anti-insulin serum, greatly diminished the rate of lactate production from glucose in jejunal segments *in vitro* and that this change was accompanied by inhibition of two key enzymes, phosphofructokinase and pyruvate dehydrogenase. The aim of the present experiments was therefore to examine whether short-term insulin deficiency alters the rate of two related processes, lipogenesis and cholesterol synthesis, which share a common precursor, acetyl-CoA, derived from pyruvate generated by intestinal glycolysis. We have used ^3H_2O to measure the rate of lipogenesis (Jungas, 1968; Brunengraber *et al.*, 1973; Stansbie *et al.*, 1976) and cholesterol synthesis (Brunengraber *et al.*, 1972; Dietschy & Spady, 1984) *in vivo* in short-term insulin deficiency induced by streptozotocin (Schein *et al.*, 1971) and after oral glucose to increase plasma insulin. Thus the findings provide direct information on the fluxes of two metabolic processes in the intestine *in vivo* in response to changes in plasma insulin. For comparative purposes, data are reported for rat liver, where lipogenesis *in vivo* is known to be sensitive to the insulin status of the rat (Stansbie *et al.*, 1976; Agius & Williamson, 1980).

EXPERIMENTAL

Female Wistar rats (210–240 g) were fed *ad libitum* on commercial chow diet PRM (E. Dixon and Sons, Ware, Herts., U.K.) containing approx. 48% (w/w) carbohydrate. They were housed in an animal house with lights on between 08:00 h and 20:30 h and maintained at a temperature of 21–24 °C. All experiments were carried out between 09:00 h and 12:00 h. Short-term insulin deficiency (Schein *et al.*, 1971) was induced by injection of 0.3 ml of streptozotocin (50 mg/ml, in 0.01 M-sodium citrate, pH 4.9) into a tail vein under ether anaesthesia (2–3 min). Where indicated, insulin-deficient rats were given a subcutaneous injection of 0.2 ml of insulin (2 units; Insulin Leo Retard; Nordisk Insulinlaboratorium, Copenhagen, Denmark). Oral glucose (2 ml; 2 M) was given to certain groups by intubation without anaesthesia. Control rats were intubated with 2 ml of water and injected with 0.3 ml of 0.9% NaCl into a tail vein. After 30 min of the various treatments, the rats were injected with ^3H_2O (0.3 ml; 5 mCi/ml) and then killed under Nembutal anaesthesia (60 mg/kg body wt.) 60 min later. Heparinized blood was collected from the abdominal aorta for the measurement of the specific radioactivity of plasma water. Liver and small intestine were excised. The latter was gently washed through with 0.9% NaCl, blotted with filter paper, adhering adipose tissue was carefully removed and two sections of about 10–12 cm were taken from the proximal end. The tissue lipids were hydrolysed under alkaline conditions (Stansbie *et al.*, 1976) and the non-saponifiable and saponifiable lipids extracted as previously described (Gibbons *et al.*, 1983). The cholesterol was separated from the non-saponifiable fraction by t.l.c. (Gibbons *et al.*, 1983). The results for the two intestinal samples were averaged to compensate for possible differences in rates of cholesterol synthesis (Feingold & Moser, 1984) and lipogenesis along the length of the intestine. Glucose was determined in whole blood by an enzymic method (Slein, 1963) after deproteinization and neutralization. Plasma insulin was measured by a double-antibody radioimmunoassay using a rat insulin standard (Albano *et al.*, 1972).

RESULTS AND DISCUSSION

Expressed per g wet wt., the rates of lipogenesis and cholesterol synthesis in intestine of control rats were about 30% of that in the livers of the same animals (Table 1). An oral load of glucose increased the rate of lipogenesis in liver by 125% ($P < 0.005$) and in intestine by 60% ($P < 0.05$; Table 1). In contrast, the rate of cholesterol synthesis was unchanged in liver and decreased by 33% ($P < 0.05$) in intestine. The increase in intestinal lipogenesis could be due to increased substrate supply and/or a raised plasma insulin. The mean plasma insulin (measured at the end of the experiment) was higher after the glucose load, but the difference was not statistically significant (Table 1); however, it is likely that the plasma insulin was significantly higher for a considerable part of the period (90 min) after the oral load. Induction of short-term insulin deficiency (90 min)

Table 1. Effects of insulin deficiency or oral glucose loading on rates of lipogenesis and cholesterol synthesis in rat intestine and liver *in vivo*

For experimental details see the text. The results are mean values ± S.E.M., with the numbers of observations in parentheses. The rates of lipogenesis or cholesterol synthesis are expressed as μmol of ^3H_2O incorporated into saponified lipid or cholesterol/h per g fresh wt. of tissue. Values that are significantly different from those of the control group by Student's *t* test are shown: $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.005$.

Experimental group	Blood glucose (mM)	Plasma insulin (munits/l)	Intestine		Liver	
			Cholesterol	Saponified lipid	Cholesterol	Saponified lipid
Control	7.00 ± 0.20 (10)	62.6 ± 11.0 (10)	0.56 ± 0.08 (10)	3.36 ± 0.27 (10)	2.04 ± 0.36 (9)	11.6 ± 1.20 (9)
Oral glucose	6.87 ± 0.37 (8)	88.9 ± 13.0 (8)	0.34 ± 0.05 (7)*	5.37 ± 0.62 (8)***	1.71 ± 0.26 (6)	26.2 ± 3.09 (8)***
Streptozotocin-treated	17.1 ± 0.45 (6)***	10.0 ± 0.9 (6)**	0.54 ± 0.05 (6)	2.53 ± 0.19 (6)*	1.03 ± 0.16 (6)*	8.21 ± 1.46 (6)*
Streptozotocin-treated + oral glucose	19.1 ± 0.40 (6)***	13.1 ± 0.8 (6)**	0.44 ± 0.05 (6)	2.00 ± 0.13 (6)***	1.01 ± 0.17 (6)*	3.46 ± 0.58 (6)***
Streptozotocin-treated + oral glucose + insulin injection	3.28 ± 0.24 (6)	—	0.28 ± 0.02 (6)**	3.86 ± 0.28 (6)	1.27 ± 0.21 (6)	16.6 ± 2.81 (6)*

with streptozotocin (Table 1; Schein *et al.*, 1971) decreased lipogenesis by 29% in liver and by 25% in intestine compared with control animals, whereas cholesterol synthesis was only decreased (50%) in liver. Administration of glucose to insulin-deficient rats did not reverse the changes induced by the lower plasma insulin; in fact there was a tendency for all values to be lower (Table 1). To check that the effects of streptozotocin treatment were due to insulin deficiency rather than a non-specific effect, a group of rats treated with the drug were injected with insulin and given oral glucose to counteract possible hypoglycaemia. In these rats the rate of liver lipogenesis was significantly higher (43%; $P < 0.05$) compared with the control rats, and the rate of intestinal lipogenesis was similar to that of the control rats (Table 1). However, intestinal cholesterol synthesis was significantly decreased (50%; $P < 0.01$), and hepatic cholesterogenesis, although not significantly different, was still lower than in control rats.

Intestinal cholesterol synthesis is increased in chronic diabetic rats (Feingold *et al.*, 1982; Goodman *et al.*, 1983), and the present acute experiments support the view that insulin deficiency does not significantly decrease the rate of intestinal cholesterogenesis in the rat (Table 1). The reason for the decreased rate of cholesterol synthesis in the intestines of rats given oral glucose or oral glucose plus insulin injection (Table 1) is not clear, although the finding implies that hyperinsulinaemia may inhibit the process. In contrast, it appears that hepatic cholesterol synthesis is decreased by short-term insulin deficiency; as far as we are aware, this has not been previously reported.

The alterations in hepatic lipogenesis *in vivo* in response to the changes in the plasma insulin concentration are in the same direction as those reported by Stansbie *et al.* (1976), who used intraperitoneal injection of glucose to vary the plasma insulin, and confirm previous work from this laboratory (Agius & Williamson, 1980). The fact that the changes in rates of lipogenesis in intestine parallel those of liver suggest that the process is also insulin-sensitive in this tissue *in vivo*, as it is in rat adipose tissue (Stansbie *et al.*, 1976) and lactating mammary gland (Robinson *et al.*, 1978; Burnol *et al.*, 1983; Jones *et al.*, 1984). The work of Kellett *et al.* (1984) indicates that acute insulin deficiency results in lower activities of phosphofructokinase and pyruvate dehydrogenase in the intestine, and these changes would be expected to decrease the availability for lipogenesis of acetyl-CoA formed from glucose. It is also possible that insulin may regulate the activity of intestinal acetyl-CoA carboxylase as it does in rat liver and adipose tissue (for review see Geelen *et al.*, 1980). In this connection, it must be emphasized that use of ^3H_2O to determine the rate of lipogenesis does not exclude the possiblity that substrates other than glucose contribute to the measured rate. A possible alternative substrate is acetate produced in the lumen by the intestinal flora (Remesy & Demigne, 1974; Buckley & Williamson, 1977).

It is concluded from the present experiments and the work of Nicholls *et al.* (1983) on the transport of glucose in the vascularly perfused jejunum that, when the rate of delivery of glucose to the intestinal lumen is high, as in oral glucose loading, the increased rate of transport of glucose via the portal blood will stimulate insulin secretion and consequently increase intestinal lipogenesis. At low rates of luminal delivery much of the available glucose will undergo glycolysis, and the resulting lactate

will be transported to the liver for glycogen synthesis or lipogenesis. In the latter case, the smaller increase in blood glucose and consequently in plasma insulin will mean a minimal increase in intestinal lipogenesis. In this way the rate of intestinal lipogenesis will be responsive to a wide range of glucose concentration in the lumen, even though the rate of provision of substrate via glycolysis is saturated at comparatively low luminal glucose concentrations (Nicholls *et al.*, 1983).

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REFERENCES

Agius, L. & Williamson, D. H. (1980) Biochem. J. **190**, 477–480
Albano, J. D. M., Ekins, R. P., Maritz, G. & Turner, R. C. (1972) Acta Endocrinol. (Copenhagen) **70**, 487–509
Bergeron, J. J. M., Rachabinski, R., Searle, N., Borts, D., Sikstrom, R. & Posner, B. I. (1980) J. Histochem. Cytochem. **28**, 824–835
Brunengraber, H., Sabine, J. R., Boutry, M. & Lowenstein, J. M. (1972) Arch. Biochem. Biophys. **150**, 392–396
Brunengraber, H., Boutry, M. & Lowenstein, J. M. (1973) J. Biol. Chem. **248**, 2656–2669
Buckley, B. M. & Williamson, D. H. (1977) Biochem. J. **166**, 539–545
Burnol, A., Leturque, A., Ferré, P. & Girard, J. (1983) Am. J. Physiol. **245**, E351–E358
Crane, R. K. (1961) Biochem. Biophys. Res. Commun. **4**, 436–440
Dietschy, J. M. & Spady, D. K. (1984) J. Lipid Res. **25**, 1469–1476
Feingold, K. R. & Moser, A. H. (1984) Am. J. Physiol. **247**, G494–G501
Feingold, K. R., Wiley, M. H., MacRae, G., Moser, A. H., Lear, S. R. & Sipperstein, M. D. (1982) Diabetes **31**, 388–395
Forgue-Lafitte, M. E., Marescot, M. R., Chamblier, M. C. & Rosselin, G. (1980) Diabetologia **19**, 373–378
Fromm, D., Field, M. & Silen, W. (1969) Am. J. Physiol. **217**, 53–57
Geelen, M. J. H., Harris, R. A., Beynen, A. C. & McCune, S. A. (1980) Diabetes **29**, 1006–1022
Gibbons, G. F., Pullinger, C. R., Munday, M. R. & Williamson, D. H. (1983) Biochem. J. **212**, 843–848
Goodman, M. W., Michels, L. D. & Keane, W. F. (1983) Proc. Soc. Exp. Biol. Med. **170**, 286–290
Jones, R. G., Ilic, V. & Williamson, D. H. (1984) Biochem. J. **223**, 345–351
Jungas, R. L. (1968) Biochemistry **7**, 3708–3717
Kellett, G. L., Jamal, A., Robertson, J. P. & Wollen, N. (1984) Biochem. J. **219**, 1027–1035
Leese, H. J. & Mansford, K. R. L. (1969) FEBS Lett. **2**, 193–194
Leese, H. J. & Mansford, K. R. L. (1971) J. Physiol. (London) **212**, 819–838
Nicholls, T. J., Leese, H. J. & Bronk, J. R. (1983) Biochem. J. **212**, 183–187
Olsen, W. A. & Rosenberg, I. H. (1970) J. Clin. Invest. **49**, 96–105
Remesy, C. & Demigne, C. (1974) Biochem. J. **141**, 85–91
Robinson, A. M., Girard, J. R. & Williamson, D. H. (1978) Biochem. J. **176**, 343–346
Schein, P. S., Alberti, K. G. M. M. & Williamson, D. H. (1971) Endocrinology (Baltimore) **89**, 827–834
Slein, M. W. (1963) in Methods of Enzymatic Analysis (Bergmeyer, H. U., ed.), pp. 117–123, Academic Press, New York and London
Stansbie, D., Brownsey, R. W., Crettaz, M. & Denton, R. M. (1976) Biochem. J. **160**, 413–416

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