Continued glucose output after re-feeding contributes to glucose intolerance in hyperthyroidism

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The effects of hyperthyroidism to elicit glucose intolerance after glucose administration were decreased under conditions where hepatic glucose output was suppressed. It is concluded that continued hepatic glucose output contributes to abnormal glucose tolerance in hyperthyroidism.

INTRODUCTION

Hyperthyroidism is associated with glucose intolerance (Kreins *et al.*, 1965). Classically, this has been suggested to result from impaired storage of carbohydrate as glycogen (Mirsky & Broh-Kahn, 1936) together with accelerated intestinal absorption (Ingbar, 1985). More recently it has become clear that this may not be correct. Orally administered glucose may be significantly metabolized by the gastrointestinal tract (Hopkirk & Bloxham, 1977). Furthermore, the synthesis of hepatic glycogen after glucose re-feeding utilizes glucose derivatives via the gluconeogenic pathway, rather than glucose directly (reviewed by Katz & McGarry, 1984).

Studies by Shikama & Ui (1978), and latterly by McGarry and co-workers (Newgard *et al.*, 1984), have demonstrated that the synthesis of liver glycogen after glucose re-feeding is coupled to suppression of hepatic glucose output. This has been confirmed by measurement of portal-venous glucose concentration differences across the liver (Holness & Sugden, 1987a). We have also observed that the normal suppression of hepatic glucose output observed after glucose ingestion is not observed in experimental hyperthyroidism (Holness & Sugden, 1987b). This finding raises the possibility that relative hyperglycaemia after re-feeding in hyperthyroidism may result from failure to redirect gluconeogenic products towards hepatic glycogen synthesis, rather than impaired glycogen storage itself.

We have applied two independent approaches to assess this possibility. First, we have inhibited gluconeogenic flux, and hence hepatic glycogen synthesis after re-feeding (Sugden *et al.*, 1983), by administration of 3-mercaptopicolinate (MPA), an inhibitor of phosphoenolpyruvate carboxykinase (DiTullio *et al.*, 1974). Second, we have investigated the effects of hyperthyroidism on hepatic glucose flux after re-feeding in the glycogen-storage-disease (*gsd/gsd*) rat, in which liver phosphorylase *b* kinase is deficient (Malthus *et al.*, 1980) and glycogen synthase *a* activity is suppressed through high glycogen concentrations (Watts & Malthus, 1980). Finally, we have investigated whether impaired peripheral glucose utilization may contribute towards hyperglycaemia in hyperthyroid rats after re-feeding. This was achieved by measuring rates of glucose disappearance after functional hepatectomy.

EXPERIMENTAL

Sources of materials were as in Sugden *et al.* (1983).

Female albino Wistar rats (*GSD/GSD*) or female (*gsd/gsd*) rats, a substrain of the NZR/Gd line, were fed *ad libitum* or starved for 48 h (in grid-bottomed cages). Rats were made hyperthyroid during the period of starvation as described by Holness *et al.* (1985).

Experiments were started between 08:30 h and 09:30 h, when glucose (2 mmol/100 g body wt.) or water (1 ml/100 g body wt.) was administered intragastrically (see Sugden *et al.*, 1983). In some experiments, MPA (20 ml/100 g body wt., in 0.15 M-NaCl, pH 7.4) or 0.15 M-NaCl was administered by intraperitoneal injection at the time of incubation (Sugden *et al.*, 1983). Rats were sampled at intervals after the intragastric load.

Rats were subjected to functional hepatectomy as described by Blackshear *et al.* (1974). This procedure (which involves ligation of the hepatic portal vein) blocks glucose release from the liver and from the intestine. The decrease in blood glucose concentration after functional hepatectomy was monitored from 0 to 15 min after surgery, with sampling from the vena cava at 4, 7, 10 and 15 min after functional hepatectomy. Rates of glucose disappearance were linear over this period. There were no changes in glucose concentrations in sham-operated controls over this period.

Glucose concentrations were determined in KOH-neutralized HClO₄ extracts of arterial, hepatic portal and hepatic venous blood by an enzymic method (Slein, 1963). Glycogen concentrations were measured in extracts of freeze-clamped liver (Keppler & Decker, 1974). Glycerol-3-phosphate dehydrogenase was measured in liver mitochondria as described by Böttger *et al.* (1970); 1 unit of enzyme converts 1 μ mol of substrate into product/min at 30 °C.

Results are given as means $\pm$ S.E.M. for 4–13 rats. Statistical significance of differences was assessed by Student's unpaired *t* test.

RESULTS AND DISCUSSION

Blood glucose concentrations and hepatic glucose output after re-feeding

Arterial glucose concentrations after the adminis-

Abbreviation used: MPA, 3-mercaptopicolinate.

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Fig. 1. Effect of hyperthyroidism on blood glucose concentrations after glucose administration to 48 h-starved rats

For experimental details see the text. $\blacktriangle$, Euthyroid rats; $\triangle$, hyperthyroid rats. Statistically significant effects of glucose administration are indicated by $**P < 0.01$, $***P < 0.001$, and of hyperthyroidism by $\dagger P < 0.05$, $\dagger\dagger\dagger P < 0.001$.

tration of glucose to 48 h-starved euthyroid and hyperthyroid rats are shown in Fig. 1. Significant effects of hyperthyroidism on glycaemia were observed during the first 90 min.

Measurement of rates of glucose disappearance after functional hepatectomy (which blocks glucose release from the liver and the intestine) indicated that hyperthyroidism was not associated with changes in extrahepatic glucose uptake after the administration of water or glucose (Fig. 2). The results show a clear correlation between the initial blood glucose concentration and the

Fig. 2. Effects of hyperthyroidism on the relationship between initial blood glucose concentration and the rate of glucose disposal after functional hepatectomy

Rats were subjected to functional hepatectomy at 1 h after the administration of water ($\triangle$, $\circ$) or at 1 h ($\blacktriangle$, $\bullet$) or 2 h ($\blacktriangle$, $\bullet$) after the administration of glucose to euthyroid ($\triangle$, $\blacktriangle$, $\bullet$) or hyperthyroid ($\circ$, $\bullet$, $\bullet$) rats. Regression analysis gave the following line equations: euthyroid, $y = -66.0 + 22.3x$ ($r = 0.79$); hyperthyroid, $y = -78.9 + 23.6x$ ($r = 0.91$). There was no significant difference between the gradients or intercepts of the two lines.

rate of decline of blood glucose after functional hepatectomy, with no effect of hyperthyroidism.

As noted previously (Holness & Sugden, 1987a), the administration of glucose to euthyroid rats led to a reversal of net glucose flux across the liver [a switch from a negative to a positive portal-minus-hepato-venous (P-V) concentration difference] (Table 1). This reversal

Table 1. Effects of hyperthyroidism on portal-hepato-venous concentration differences [(P-V)], hepatic glycogen concentrations, and arterial glucose concentrations after re-feeding in normal or phosphorylase kinase-deficient (*gsd/gsd*) 48 h-starved rats

Rats were sampled at 1 h after the administration of water or glucose by intragastric intubation. Results are means $\pm$ S.E.M. for 4-13 rats. Statistically significant effects are indicated: $^aP < 0.05$, $^bP < 0.01$, $^cP < 0.001$ for control versus hyperthyroid; $^dP < 0.05$, $^eP < 0.01$, $^fP < 0.001$ for water versus glucose; $^gP < 0.05$, $^hP < 0.01$, $^iP < 0.001$ for NaCl versus MPA or for *GSD/GSD* versus *gsd/gsd*.

	(P-V) (mm)		Glycogen (g/100 g of liver)		Arterial [glucose] (mm)	
	Control	Hyperthyroid	Control	Hyperthyroid	Control	Hyperthyroid
Expt. I						
NaCl + water	-0.48 ± 0.08	-0.73 ± 0.12	0.09 ± 0.02	0.12 ± 0.04	5.18 ± 0.19	5.77 ± 0.28
NaCl + glucose	$+0.53 \pm 0.11^f$	-0.15 ± 0.12^{bc}	0.84 ± 0.17^f	0.37 ± 0.08^{ad}	7.48 ± 0.28^f	11.21 ± 0.49^{cf}
MPA + water	-0.14 ± 0.03^h	-0.01 ± 0.27^g	0.14 ± 0.03	0.18 ± 0.07	1.66 ± 0.16^i	1.61 ± 0.20^i
MPA + glucose	$+0.67 \pm 0.32^d$	$+1.38 \pm 0.66^{dg}$	0.29 ± 0.10^g	0.23 ± 0.05	8.67 ± 0.55^f	8.36 ± 0.72^{th}
Expt. II						
<i>GSD/GSD</i> + water	-0.48 ± 0.08	-0.74 ± 0.11	0.08 ± 0.04	0.12 ± 0.03	4.80 ± 0.18	5.28 ± 0.40
<i>GSD/GSD</i> + glucose	$+0.69 \pm 0.13^e$	-0.41 ± 0.18^c	0.69 ± 0.10^e	0.44 ± 0.05^{ac}	7.33 ± 0.19^e	11.95 ± 0.27^{ce}
<i>gsd/gsd</i> + water	-0.39 ± 0.12	-0.23 ± 0.07	12.91 ± 0.58^i	13.44 ± 0.34^i	4.12 ± 0.23^g	4.82 ± 0.17^a
<i>gsd/gsd</i> + glucose	$+0.96 \pm 0.30^d$	$+1.35 \pm 0.21^{ei}$	12.61 ± 0.30^i	12.61 ± 0.30^i	5.98 ± 0.26^i	7.04 ± 0.41^{af}

was not observed if the rats were hyperthyroid. As the most significant effects of glucose re-feeding on hepatic glucose uptake are observed at early time points (Holness & Sugden, 1987a), and furthermore, in the present experiments effects of hyperthyroidism on glycaemia were also more apparent at these times (Fig. 1), subsequent experiments utilized a 1 h re-feeding period.

As shown in Table 1, and demonstrated previously (Okajima & Ui, 1979; Sugden *et al.*, 1981), glycogen synthesis was impaired in hyperthyroidism. Bollen & Stalmans (1987) have shown that this effect is not mediated via a diminished response of glycogen synthase to glucose administration.

Effects of inhibition of gluconeogenesis (Expt. I)

(i) **Water-re-fed rats.** The administration of MPA to starved (water-re-fed) rats decreased hepatic glucose output, as assessed by portal-minus-hepato-venous concentration differences [(P–V)] (Table 1). Glycogen concentrations were low and unchanged by MPA. Marked hypoglycaemia was observed. These effects of MPA were apparent in both euthyroid and hyperthyroid rats (Table 1). These results confirm that hepatic gluconeogenesis makes a major contribution to blood glucose in the starved state.

(ii) **Glucose-re-fed rats.** MPA did not affect (P–V) after re-feeding in euthyroid rats, but glycogen synthesis was inhibited (Table 1). Arterial glucose concentrations were unchanged (Table 1). These findings demonstrate that, consistent with suppression of glucose output, liver gluconeogenesis does not normally contribute to glycaemia after re-feeding, and confirm that hepatic glycogen synthesis uses precursors metabolized via the gluconeogenic pathway rather than glucose itself. Notably, in MPA-treated rats, such selective utilization of precursors occurs even in the face of net glucose uptake, indicated by a positive value of (P–V).

A positive value of (P–V) also indicated net hepatic glucose uptake in hyperthyroid rats given glucose in combination with MPA (Table 1). Hepatic glycogen and, importantly, arterial glucose concentrations were decreased (Table 1). Consequently, in the presence of MPA, neither (P–V) nor glycogen or blood glucose concentrations were affected by hyperthyroidism.

It is interesting that, although MPA inhibits gluconeogenesis after re-feeding, net hepatic glucose uptake is observed. It may therefore be inferred that any glucose 6-phosphate derived directly from glucose is directed into pathways other than glycogen synthesis. In the euthyroid state, glycolysis is restricted at the level of 6-phosphofructokinase-1 through low fructose 2,6-bisphosphate concentrations (Kuwajima *et al.*, 1984), but there is significant flux through the pentose phosphate pathway (Scofield *et al.*, 1985). Such flux may be increased by MPA treatment as a consequence of increased lipogenesis (Sugden *et al.*, 1983), with concomitant increases in NADPH demand.

Effects of phosphorylase *b* kinase deficiency (Expt. II)

(i) **Water-re-fed rats.** As noted previously (Clark *et al.*, 1985), liver glycogen concentrations in *gsd/gsd* rats were only slightly lower than those found in the fed (euthyroid) state (15.58 ± 0.40 g/100 g of liver), and 3 times those of fed *GSD/GSD* rats, despite starvation for 48 h. There

was no significant effect of hyperthyroidism on hepatic glycogen content in starved *gsd/gsd* rats, indicating that active phosphorylase *b* kinase is a prerequisite for glycogen depletion in response to the hormone. Blood glucose concentrations were lower than those found in *GSD/GSD* rats, but only marginally so, and, consistent with the idea that glycaemia is maintained by hepatic glucose output (Malthus *et al.*, 1980; Clark *et al.*, 1985), a negative value of (P–V) indicated glucose release in both euthyroid and hyperthyroid *gsd/gsd* rats.

(ii) **Glucose-re-fed rats.** Glucose administration led to net hepatic glucose uptake [a positive (P–V)] not only in euthyroid but also in hyperthyroid rats. The biochemical basis for the different hepatic responses of hyperthyroid *GSD/GSD* and *gsd/gsd* rats remains to be established. The efficacy of thyroid-hormone treatment (assessed by the induction of hepatic mitochondrial glycerol-3-phosphate dehydrogenase) was unchanged [*GSD/GSD*: control, 0.44 ± 0.06 ; hyperthyroid, 1.44 ± 0.16 (units/g); *gsd/gsd*: control, 0.55 ± 0.09 ; hyperthyroid, 1.38 ± 0.13 (units/g); $P < 0.001$ in each case]. As expected from the suppression of glycogen synthase *a* activity by glycogen in *gsd/gsd* rats, glycogen concentrations were unaffected by re-feeding. Differences in glycaemia between euthyroid and hyperthyroid rats, although still observed, were greatly decreased in the *gsd/gsd* rats. Moreover, the increments in glycaemia after glucose re-feeding were similar in both groups.

Conclusions

The effects of hyperthyroidism to increase glycaemia after glucose administration were no longer evident or were greatly decreased when hepatic glucose output was suppressed through inhibition of gluconeogenesis (Expt. I) or glycogen breakdown (Expt. II). It may be concluded that the continued glucose efflux from the hyperthyroid liver makes a major contribution to glycaemia after re-feeding.

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