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INTERRELATION BETWEEN THYROID STATE AND THE EFFECT OF GLUCAGON ON GLUCONEOGENESIS IN PERFUSED RAT LIVERS

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Abstract—The effects of different thyroid states on glucagon/dBcAMP-induced gluconeogenesis from alanine or lactate were investigated in the isolated perfused liver from 24-hr starved rats. (1) Gluconeogenesis from alanine varied with the thyroid state, being increased in hyperthyroidism and decreased in hypothyroidism. (2) Both glucagon and dBcAMP increased glucose production from alanine in euthyroid and even less pronounced in hypothyroid livers, the effect was dose dependent; concomitantly alanine and [^{14}C] α-amino-isobutyric acid uptake increased. In hyperthyroid liver, both glucagon and dBcAMP stimulated neither hepatic uptake of alanine and [^{14}C] α-amino-isobutyric acid nor gluconeogenesis from alanine. (3) Lactate uptake as well as glucose production from lactate varied with the thyroid state, being increased in the hyper- and decreased in the hypothyroid state. (4) Both glucagon and dBcAMP increased lactate uptake as well as gluconeogenesis from lactate: the effect was even more pronounced in hyperthyroid and reduced in hypothyroid liver.

We conclude that the glucogenic effect of glucagon/dBcAMP is reduced in the hypo- and—at unlimited substrate supply—stimulated in the hyperthyroid liver.

Hyper- and hypothyroidism are not only characterized by increased or decreased concentrations of thyroid hormones: tissue responsiveness and/or alterations in the serum concentrations of other hormones have been reported, e.g. catecholamines, insulin, glucagon, steroids [1–5]. Thus thyroid hormone action on intermediary metabolism is considered to be—at least in part—mediated by other hormones [1, 6]. However, with respect to glucagon this issue is controversial: its effect on hepatic glucose metabolism is reported to be increased in hyperthyroidism [7], while other workers provide evidence that thyroid hormones blunt the glucogenic effect of glucagon *in vivo* and *in vitro* [8–10]. As different thyroid states have profound effects on hepatic gluconeogenesis [1, 3, 11], this paper sets out to investigate the effect of glucagon on hepatic gluconeogenesis in hypo- and hyperthroidism.

MATERIALS AND METHODS

Male Wistar (specific pathogen-free) rats (180–210 g) were housed under controlled conditions. Different thyroid states were induced as described previously [12]: hypothyroidism radiochemically (250 μCi ^{131}I/100 g b.w. 28–38 days before the experiment, serum T4 < 10 ng/ml)‡ and hyperthyroidism by daily T4 injection (50 μg T4/100 g b.w. intraperitoneally for 8 days before the experiments increasing serum T4 to >200 ng/ml). The "organ specific" thyroid state was controlled by measurement of hepatic malic enzyme activity, which was estimated to be <2 mU/mg protein in hypo- and >40 mU/mg protein in hyperthyroid rats. All experiments were performed in 24-hr-starved rats in order to deplete hepatic glycogen stores (hepatic glycogen amounted to 1.60 ± 0.36 in the hypo-, 1.43 ± 0.26 in the eu- and 1.26 ± 0.36 μmoles glycosyl units/g liver wwt in the hyperthyroid state, N = 6 for each thyroid state).

Livers were isolated and perfused as described previously [12]. The functional state of the livers was controlled by repeated measurement of perfusate pH (7.35), hepatic O_2-consumption (2.0 ± 0.2 in the hypo-, 3.3 ± 0.2 in the eu-, and 3.9 ± 0.2 μmoles/g liver wet wt × min in the hyperthyroid state, N = 8–12) and glutamate oxaloacetate transaminase release (less than 2.5 U/g liver wet wt in 120 min). The first 50 ml of perfusate passing through the liver were discharged, experiments were started at zero time after a 30 min equilibration period. The initial perfusate (Fluorocarbon/FC 43, cf. [12]) contained 2.8 mM glucose, 10 mM alanine or lactate; the gluconeogenic precursors were added continuously in order to avoid substrate depletion (15 μmol/min, each). Glucagon or dBcAMP were added at zero time as indicated in the legends. In separate experiments AIB uptake was estimated after addition of AIB (final concentration 0.1 mM) and 2 μCi α-amino[^{14}C]isobutyric acid to the medium. In some

‡ Abbreviations used: T3, L-3,3',5-tri-iodothyronine; T4, L-thyroxine; AIB, α-amino-isobutyric acid; MIX, 1-methyl-3-isobutyl-xanthine.

experiments MIX was given to the perfusate (final concentration 50 μM) at zero time.

Metabolic flux rates, amino acid uptake, oxygen consumption, glutamate oxaloacetate transaminase activity in the medium as well as the tissue level of cAMP were determined as described previously [12]. For the estimation of malic enzyme activity a small liver lobe was removed immediately after liver preparation.

Buffer substrates were purchased from E. Merck (Darmstadt). All other substrates, cosubstrates, nucleotides and coupling enzymes needed for the determination of enzyme activities, metabolites and cAMP were obtained from C. F. Boehringer (Mannheim); AIB, MIX, T4 and T3 were from Sigma Chemicals (Munich); highly purified porcine glucagon from Novo Research Institute (Bagsvaerd, Denmark); [^{14}C]α-amino-isobutyric acid was bought from New England Nuclear Corporation.

The statistical significance of differences were examined using Student's *t*-test and, where appropriate, by analysis of variance.

RESULTS

Net glucose output from alanine, net alanine uptake, net urea production (Fig. 1 and Table 1)

Net glucose output from alanine varied with the thyroid state being increased in hyper- (significance vs euthyroid: $P < 0.01$) and decreased in hypothyroid perfused liver (significance vs euthyroid: $P < 0.05$). Glucagon as well as dBcAMP were capable of increasing glucose output from alanine in eu- and slightly less pronounced in hypothyroid liver, but were without effect in the hyperthyroid liver.

Net alanine uptake and urea production varied with the thyroid state. Both were increased in hyper- (significance vs euthyroid: $P < 0.01$) and decreased in hypothyroid perfused livers (significance vs euthyroid $P < 0.05$). Both glucagon and dBcAMP increased alanine uptake as well as urea production in hypo- and euthyroid livers, but were without effect in the hyperthyroid state. Calculation of the possible alanine conversion into glucose (derived from urea production, cf. Table 1 and assuming 100% conversion efficiency, i.e. 100% of alanine is converted to glucose) revealed a conversion efficiency of 32%

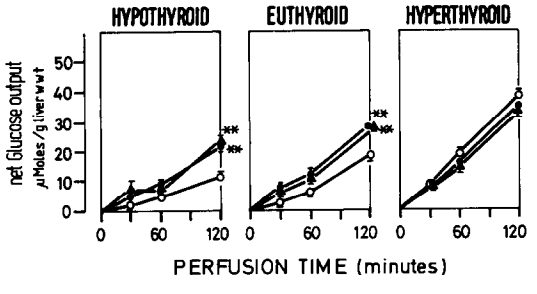

Fig. 1. Effect of glucagon (●) or dBcAMP (▲) (controls, ○) on net glucose output from alanine in the isolated perfused liver from hypo-, eu- and hyperthyroid starved rats. Initial concentration of glucagon 10^{-8} M, of dBcAMP 2×10^{-4} M. Data are given as means ± SEM (N = 8–13). Significance: **$P < 0.01$ vs controls.

Table 1. Effect of glucagon or dBcAMP on AIB uptake, net alanine uptake and urea production in the isolated perfused liver from hypo-, eu- and hyperthyroid starved rat

	Thyroid state								
	Hypothyroid			Euthyroid			Hyperthyroid		
Addition:	—	+Glucagon	+dBcAMP	—	+Glucagon	+dBcAMP	—	+Glucagon	dBcAMP
AIB uptake†	0.25 ± 0.03	0.42 ± 0.04†	0.38 ± 0.03*	0.26 ± 0.03	0.37 ± 0.03*	0.40 ± 0.04†	0.40 ± 0.04	0.39 ± 0.04	0.43 ± 0.04
Net alanine uptake†	1.0 ± 0.1	1.7 ± 0.2*	1.9 ± 0.1†	1.1 ± 0.1	1.7 ± 0.1†	1.8 ± 0.1†	2.2 ± 0.1	1.9 ± 0.1	2.1 ± 0.1
Urea production†	0.25 ± 0.01	0.36 ± 0.01	0.33 ± 0.02†	0.30 ± 0.02	0.40 ± 0.02*	0.40 ± 0.02†	0.43 ± 0.02	0.35 ± 0.01	0.38 ± 0.01

† AIB uptake in μmoles/g liver wet wt × 120 min, net alanine uptake and urea production in μmoles/g liver wet wt × min. Initial concentration of glucagon 1×10^{-8} M and of dBcAMP 2×10^{-4} M. Data are given as means ± SEM (N = 8–12). Significance: *$P < 0.05$, †$P < 0.01$.

in hypo-, 53% in eu-, and 71% in hyperthyroid livers (cf. data in Table 1 and Fig. 1). The conversion efficiency was increased by glucagon/dBcAMP to 50%/51% in hypo-, and 62%/62% in eu-, yet was without effect in hyperthyroid livers (71%/74%).

AIB uptake (Table 1)

AIB uptake was significantly increased in hyperthyroid liver (significance $P < 0.01$ vs euthyroid control), no difference between hypo- and euthyroidism was observed. Glucagon as well as dBcAMP were capable to increase [^{14}C]-AIB uptake in hypo- and euthyroidism, but were without effect in the hyperthyroid state.

Concentration dependency for glucagon (Fig. 2)

Glucagon increased glucose output from alanine in hypo- and euthyroid livers. This effect was dependent on the concentration, but was less pronounced in the hypothyroid state. No effect was observed for hyperthyroid liver. In euthyroidism, glucagon elevated hepatic cAMP. In hypothyroidism, the concentration dependent curve was shifted to the right. In hyperthyroid liver basal tissue cAMP was significantly enhanced when compared to the euthyroid value ($P < 0.01$). Increasing glucagon did not further increase the concentration of the cyclic nucleotide.

Concentration dependency for dBcAMP (Fig. 3)

Increasing dBcAMP increased net glucose output from alanine in euthyroid livers. In hypothyroidism the stimulatory effect of the second messenger on glucose output was less pronounced, but was blunted in hyperthyroidism. Addition of sodium butyrate was without effect on all parameters measured.

Inhibition of phosphodiesterase activity by MIX

Inhibition of phosphodiesterase activity by MIX in the presence of glucagon (10^{-8} M) resulted in a similar maximal tissue cAMP level (hypo-: 1.4 ± 0.1, eu-: 1.5 ± 0.1 and hyperthyroid: 1.3 ± 0.1 nmoles/g liver wet wt) independent of the thyroid state. Despite this effect no significant differences in net alanine uptake and net glucose output from alanine were observed when compared to presence of glucagon alone.

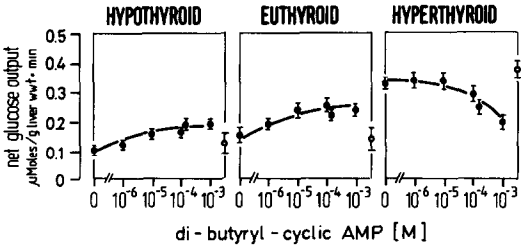

Fig. 3. Effect of increasing concentrations of dBcAMP (●) in the perfusate on net glucose output from alanine in the isolated perfused liver from hypo-, eu- and hyperthyroid starved rat. Controls received 1×10^{-3} M sodium butyrate (○). Initial concentrations of dBcAMP are depicted, data are given as means ± SEM (N = 3–6).

Net glucose output from lactate (Fig. 4)

Net glucose output from lactate varied with the thyroid state being increased in hyper- ($P < 0.01$ vs euthyroid control) and decreased in hypothyroidism ($P < 0.01$ vs euthyroid control). Both glucagon and dBcAMP were capable of increasing glucose output from lactate in all thyroid states; compared to controls this effect was less pronounced in the hypo (Δ increase: eu- vs hypothyroid liver $P < 0.05$), yet more

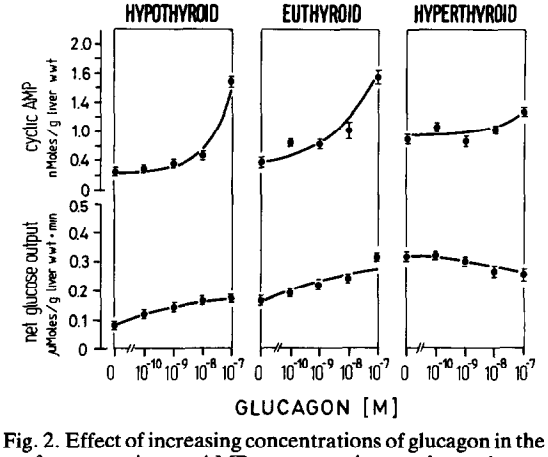

Fig. 2. Effect of increasing concentrations of glucagon in the perfusate on tissue cAMP concentration, and net glucose output from alanine in the isolated perfused liver from hypo-, eu- and hyperthyroid starved rats. cAMP levels were measured at 120 min perfusion time, initial concentrations of glucagon are depicted. Data are means ± SEM (N = 3–6). Statistical significance for the increase in cyclic AMP: hypothyroid liver control value versus glucagon 10^{-10} M n.s., versus 10^{-9} M, $P < 0.05$, versus 10^{-8} M and versus 10^{-7} M, $P < 0.01$. Increase in hepatic cyclic AMP in hypo- and euthyroid liver $P < 0.01$, in hyperthyroid liver n.s.

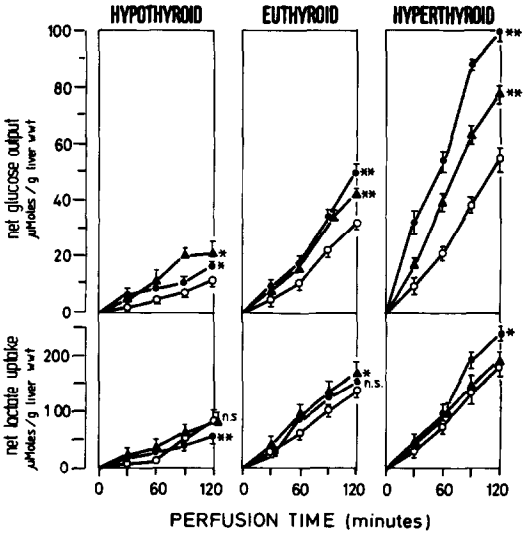

Fig. 4. Effect of glucagon (●) or dBcAMP (▲) (controls, ○) on net lactate uptake and net glucose output from lactate in the isolated perfused liver from hypo-, eu- and hyperthyroid rats. Initial concentration of glucagon 1×10^{-8} M, of dBcAMP 2×10^{-4} M. Data are given as means ± SEM (N = 4). Significance vs controls: *$P < 0.05$, **$P < 0.01$.

pronounced in the hyperthyroid state (Δ increase eu- vs hyperthyroid liver: $P < 0.01$). Concomitantly lactate uptake varied with the thyroid state being increased in the hyper- ($P < 0.05$ vs euthyroid control) and reduced in the hypothyroid liver ($P < 0.01$ vs euthyroid control). Both glucagon and dBcAMP were without significant effects on hepatic net lactate uptake in eu- and hypothyroidism, but glucagon significantly increased lactate uptake in hyperthyroidism.

Gluconeogenic key enzymes

The activities of the gluconeogenic key enzymes in hypo-, eu- and hyperthyroid liver were as follows: pyruvate carboxylase 63 ± 6, 105 ± 8, 173 ± 5, P-enolpyruvate carboxykinase 118 ± 9, 144 ± 2, 201 ± 13, and fructose 1, 6 biphosphatase 108 ± 14, 177 ± 2, 176 ± 3 mU/mg protein (means ± SEM, $N = 6$). Within 2 hr perfusion time neither glucagon (1×10^{-8} M) nor dBcAMP (2×10^{-4} M) affected these enzyme activities.

DISCUSSION

The present data provide some insight into the hormonal regulation of hepatic gluconeogenesis in hypo- and hyperthyroidism. Thyroid hormones increase hepatic glucose production by at least four different mechanisms: (i) directly by increasing gluconeogenic precursor (i.e. alanine) supply within minutes [12–14]; (ii) directly by increasing cytosolic ATP-availability within 20–30 min [15, 16]; (iii) by regulating the transfer of reducing equivalents across the mitochondrial membrane [17, 18]; and (iv) by increasing the synthesis and activity of gluconeogenic key enzymes, e.g. P-enolpyruvate carboxykinase, within hours without affecting its degradation rate [19–21]. Accordingly, hepatic gluconeogenesis is decreased in hypo- and increased in hyperthyroid rat *in vivo* and *in vitro* (cf. [1]).

The accelerated rate of glucose production observed in hyperthyroidism has been ascribed to a substantial part to the considerably elevated levels of serum glucagon [7, 13, 22–24] which consequently should lead to an enhanced tissue cAMP content. In fact measurement of hepatic cAMP levels revealed increased values under this endocrine condition [12, 13, 18] (Fig. 2). As T3 by itself increased neither hepatic cAMP level nor cAMP dependent protein kinase activation as measured in the isolated perfused liver [14], this increase is not due to a direct effect of thyroid hormones. In addition comparing the effect of glucagon on hepatic gluconeogenesis in hypo- and hyperthyroid liver it is evident from our results (Fig. 4) that in the latter glucagon was significantly more effective. This finding contrasts to the lacking glucagon effect on hepatic glycogenolysis and ketogenesis in the liver from hyperthyroid animals [8, 25–27]. Addition of glucagon was followed by a corresponding increase in tissue cAMP in eu- and a delayed increase in hypothyroid livers (Fig. 2), whereas the effect of glucagon on stimulating gluconeogenesis (from lactate) in the hyperthyroid liver occurred at virtually unaltered tissue cAMP levels (Fig. 2). Correspondingly, a blunted response to epinephrine and glucagon in cAMP output was shown for hepatocytes isolated from T3-treated rats [28]. Therefore it is tempting to speculate that the observed glucagon-mediated stimulation of gluconeogenesis (from lactate) is due to other than cAMP-mediated effects of glucagon (e.g. on the mitochondrial compartment) [29].

In hypothyroid liver increased activities of phosphodiesterase were observed [30, 31], favouring the stimulation of hepatic cAMP degradation and thereby explaining the reduced glucagon response [30]. However, in hypothyroid livers inhibition of phosphodiesterase by MIX in the presence of glucagon had only a small statistically insignificant effect on the rate of gluconeogenesis, questioning the metabolic relevance of increased phosphodiesterase activity in the hypothyroid state.

In hypo- and euthyroid liver, glucagon and dBcAMP stimulated glucose production from alanine in a dose dependent manner (Figs 2 and 3). However, in the perfused liver isolated from hyperthyroid animals no effect of glucagon was observed (Fig. 1). At first sight this would confirm the suggestion of others that the adenylate cyclase system shows an impaired function in hyperthyroidism [28] (cf also Fig. 2). However, circumvention of the receptor adenylate cyclase system by addition of dBcAMP demonstrated a similar response (Fig. 3). The failure of both glucagon and dBcAMP to stimulate alanine conversion to glucose is explained by our results measuring alanine uptake and the capacity of the alanine transport system of the liver cell membrane by AIB uptake measurement (Table 1). Both net alanine and AIB uptake are already maximally elevated in hyperthyroid liver and this could not be further stimulated by glucagon: consequently further elevation of gluconeogenes is due to "limited" intracellular substrate supply. This is even documented by urea production from alanine which remained unaffected by glucagon in hyperthyroid liver (Table 1). Recently it has been suggested that in hyperthyroidism gluconeogenesis from pyruvate (and consequently alanine) may be limited by supply of reducing equivalents which favour the glycerinaldehyde-P-dehydrogenase reaction in the cytosol [17]. This explanation seems unlikely: (i) addition of alanine to the perfusate of the hyperthyroid liver increased and not decreased the cytosolic redox potential as indicated by the ratio of liver lactate/pyruvate [12]; (ii) addition of ethanol to the perfusate, providing reducing equivalents in the liver cell, had no additional effect on alanine conversion into glucose in hyperthyroid liver [32].

Our data demonstrate that the effect of glucagon on hepatic gluconeogenesis is reduced in hypo- and with respect to lactate increased in hyperthyroidism, whereas glucagon fails to stimulate alanine conversion into glucose in hyperthryoid rats. Taken together our data provide evidence that—at unlimited substrate supply—increased gluconeogenesis observed in hyperthyroidism is in part due to the action of glucagon.

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