Experimental hyperthyroidism does not induce hepatic insulin resistance in the miniature pig

Manfred J. MÜLLER,* Jasper MÖRING† and Hans J. SEITZ†
*Medizinische Hochschule Hannover, Abteilung Klinische Endokrinologie, Hannover, and †Universitäts-Krankenhaus Eppendorf, Institut für Physiologische Chemie, Hamburg, Federal Republic of Germany

The effect of hypo- and hyper-thyroidism on insulin-mediated alterations in tracer-determined glucose kinetics and the arterial concentration of gluconeogenic precursors were investigated in 24-h-starved conscious unrestrained miniature pigs. Hyperinsulinaemia (about 40 μunits/ml) decreased blood glucose and, transiently, glucose output at unaltered glucose utilization in all thyroid states: this effect was pronounced in hyperthyroid (−50%) and less in hypothyroid pigs (−25%) compared with euthyroid controls (−35%). We conclude that moderate experimental hyperthyroidism does not induce hepatic insulin resistance, whereas hypothyroidism slightly impairs insulin action with respect to the regulation of glucose output.

INTRODUCTION

The interaction of insulin and thyroid hormones on the regulation of glucose metabolism is still a matter of debate (for reviews, see Bratusch-Marrain (1983), Lenzen & Bailey (1984) and Müller & Seitz (1984]). Thyroid hormones are generally considered to antagonize the action of insulin on glucose homoeostasis. This has been based mainly on several clinical studies showing an impaired oral glucose-tolerance curve at concomitantly elevated insulin concentrations in hyperthyroid patients. In fact, by using the more sophisticated insulin-sensitivity test a decrease in the response to insulin in hyperthyroid patients was shown (Yasuda et al., 1984; Shen & Davidson, 1985). Furthermore, an antagonism between insulin and thyroid hormones was reported for the regulation of hepatic glucose production (Bratusch-Marrain et al., 1984; Dimitriadis et al., 1985; Laville et al., 1984).

In contrast, in experimental hyperthyroidism no peripheral insulin resistance could be observed (Laville et al., 1984; Dimitriadis et al., 1985). Accordingly, insulin sensitivity as measured by the euglycaemic-clamp technique at 40 μunits of insulin/ml revealed no differences between eu- and hyper-thyroid subjects (McCulloch et al., 1983b). Surprisingly, as measured by the hyperglycaemic-clamp technique, exposure to T₃ in healthy man even increased insulin-induced glucose utilization (Bratusch-Marrain et al., 1984).

On the basis of these discrepancies, the present study investigated the effect of physiological hyperinsulinaemia on glucose kinetics by a different approach: (i) with a standardized animal model, the conscious, unrestrained, hypo-, eu- and moderately hyper-thyroid miniature pig, (ii) by applying the sensitive tracer technique for measurement of glucose production and utilization.

METHODS

Animals

In conducting the research described in this report, the investigators adhered to the ‘Guiding Principles in the Care and Use of Animals’ approved by the council of The American Physiological Society. The facilities are fully accredited by the ‘Gesundheitsbehörde der Freien und Hansestadt Hamburg’; over the whole experimental period, veterinary supervision of the animals was supplied.

Some 15 male castrated miniature pigs (specific-pathogen-free; 28–32 kg) were purchased (Versuchsgut, Domäne Reilliehausen, Göttingen, Germany) 8 weeks before starting the experiments. Animals were kept under controlled conditions: 23 °C room temperature, 60% humidity, 12 h-light/12 h-dark cycle providing light from 07:00 to 19:00 h. The animals were kept on a standard chow diet (Ibeka Kraftfutter, Hamburg, Germany; ingredients (w/w): 57% starch, 5.5% glucose, 16% crude protein, 7% minerals and trace elements, 5.3% fibre, 7% vitamin mixture and Tylosin (10 mg/kg); total energy value 12950 kJ/kg), receiving 300 g/day in one portion at 07:00 h. Different thyroid states were produced as described briefly: hypothyroidism by surgical thyroidectomy followed by radiochemical treatment (16 mCi of ¹³¹I/pig) 6–7 weeks before starting the experiments, leading to non-measurable serum T₄ and T₃ concentrations at least 4 weeks after treatment. Experimental hyperthyroidism resulted from a daily T₃ (Sigma) treatment for at least 10 days (5.0 μg/kg body wt.), leading to elevated serum T₄ and T₃ values (T₄ > 130 ng/ml, T₃ > 1.8 ng/ml) compared with euthyroid animals (T₄ = 36 ± 3 ng/ml, T₃ = 1.3 ± 0.1 ng/ml) (cf. Müller et al., 1983b).

Experimental procedures

Polyvinyl catheters were implanted into the aortic arch, the vena cava superior and one vena jugularis externa 8 days before the experiments. The recovery from surgery was checked by measurement of serum protein, glutamate: oxaloacetate transaminase activity, serum glutamate: pyruvate transaminase activity, body weight and body temperature (Paschen et al., 1982; Müller et al., 1983a,b).

All experiments were performed after 24 h starvation; hepatic glycogen stores were 27 ± 3 and 131 ± 9 μmol of...
glycosyl units/g wet wt. of liver in eu- and hyperthyroidism respectively, measured in separate experiments in which liver biopsies were performed under anaesthesia (n = 4 for each experimental condition). A primed (20 μCi) continuous (0.20 μCi/min) infusion of [3-3H]glucose (1 mCi/ml; New England Nuclear Corp., Dreieich, Germany) was given via the vena cava superior for isotopic determination of the rates of glucose production and utilization. Up to 120 min was allowed for isotope equilibration; steady state was reached usually between −20 min and zero time, as was reflected by the constancy in the concentration of labelled and unlabelled compounds in the blood. Blood samples were drawn from the arterial line from −120 to −110 min each min, then at −110, −100 and −90 min (for determination of the distribution volume), and then from −20 to 0 min at 5 min intervals. During and after the infusion period blood samples were drawn at 15 min intervals.

Analytical methods

Methods for the determination of hormones and metabolites have been described previously by Müller et al. (1983a,b). Plasma immunoreactive insulin was estimated by using pig insulin for preparation of the standard curve; for the measurement of iodothyronines, a thyronine-free pig serum was prepared as in Müller et al. (1983b). Plasma immunoreactive glucagon was measured by using Unger’s 30 K antibody, by using pig glucagon (Novo Research, Copenhagen, Denmark) for the preparation of the standard curve, and following the instructions in the kit manual.

Labelled glucose was determined as in Müller et al. 1986.

Fig. 1. Effect of continuous infusion of insulin (0.4 munits/min per kg body wt. for 6 h) on arterial serum insulin, plasma glucagon, blood glucose, the rate of endogenous glucose production (Ra) and utilization (Rd) in conscious 24 h-starved hypo-, eu- and hyper-thyroid miniature pigs

Data are given as means ± S.E.M. (n = 5). Statistical significance for the alterations induced by the insulin infusion: increase in serum insulin (P < 0.01 in all thyroid states); increase in plasma glucagon (euthyroid P < 0.01; hypo- and hyper-thyroid not significant); decrease in blood glucose (P < 0.01 in all thyroid states); alterations in Ra (eu- and hypo-thyroid P < 0.05; hyperthyroid not significant); alteration in Rd (not significant in all thyroid states).
Hepatic insulin response in hypo- and hyper-thyroidism

(1983a, 1984), briefly: 0.5 ml blood samples were deproteinized by adding 1 ml of 5.35% (w/v) Ba(OH)\textsubscript{2}, 1 ml of 5% (w/v) ZnSO\textsubscript{4},7H\textsubscript{2}O and 5 ml of distilled water. After centrifugation, a 1 ml sample of the supernatant was evaporated to dryness under vacuum at −70 °C; the residue was redissolved in 1 ml of water. Bray’s scintillant was added and radioactivity was counted in a Berthold liquid-scintillation spectrophotometer. The counting efficiency of \(^3\)H was approx. 35%; the recovery of standards was 94%. Measurements were done in triplicate.

Plasma volume was estimated from single injection of Indocyanine Green (Hynson, Westcott and Dunning, Baltimore, MD, U.S.A.). Blood samples were taken at 0, 4, 6, 8 and 10 min, the dye was measured immediately at 805 nm in a Zeiss PMQ III spectrophotometer, and the dye concentration was read from a standard curve prepared for each animal; plasma turbidity was avoided by addition of 5 g of deoxycholate/100 ml (Müller et al., 1983a; Paschen et al., 1982). The measurement of plasma volume in three hypo-, eu- and hyper-thyroid animals revealed a value of 2200–2500 ml/25 kg pig.

Calculations

The rates \(R_a\) and \(R_d\) were calculated from the equations of Steele (1959) in their derivative form (Cowan & Hetenyi, 1971). In the basal state, when a dynamic equilibrium prevailed, the rate of glucose turnover \(R_a\) was calculated by the isotope-dilution equation: \(R_a = F/SA\) (\(F\), the rate of infusion of the tracer; \(SA\), specific radioactivity of glucose). Under this condition, \(R_t = R_a = R_d\). In the non-steady state, \(R_a\) and \(R_d\) were calculated from

\[ R_a = \left( F - p \cdot V_c \cdot (dSA/dt) \right) \div SA_t \]

and

\[ R_d = p \cdot V_c \cdot (dc/dt), \]

where \(c\) is the concentration of glucose and \(V\) represents the glucose distribution volume, which was determined for each animal by fitting the early time course (−120 to −90 min) from labelled glucose to a single pool model of glucose. \(P\) is the pool fraction, which reflects a factor to compensate for non-ideal pool behaviour. Although it is impossible to determine the optimum correction factor [for details, cf. Wolfe (1984)], the following pragmatic approach was performed. Plasma volume, as determined from single Indocyanine Green injection (see above), was divided by the glucose distribution volume. Values between 0.58 and 0.72 were obtained, on average 0.65. No significant differences were found for the different thyroid states. Thus a correction factor of 0.65 was applied, which is similar to a value applied by Cowan & Hetenyi (1971).

Presentation of results and statistics

The data are presented as means ± S.E.M. Their statistical significance was evaluated either by analysis of variance or by two-tailed unpaired and, when appropriate, paired \(t\) test.

RESULTS

As shown previously (Müller et al., 1983b), hyperthyroid miniature pigs showed increased blood glucose as well as increases in \(R_a\) and \(R_d\) (\(P < 0.05, P < 0.01\) and \(P < 0.01\) respectively), whereas glucose kinetics were unaffected by thyroidectomy (Fig. 1). Plasma insulin concentration did not differ between the thyroid states, whereas glucagon (30 K-immunoreactive) was significantly increased in hyperthyroid pigs (\(P < 0.05\)). Increasing insulin to similar steady-state values (about 40 μ units/ml) enhanced plasma 30 K-immunoreactive glucagon in euthyroidism (\(P < 0.05\)), but had no significant effect in hypo- and hyper-thyroidism (Fig. 1). As a consequence of insulin infusion, blood glucose decreased in all thyroid states (Fig. 1): compared with euthyroidism (\(Δ0–120\) min −1.77 ± 0.22 mm), the effect was less pronounced in hypothyroidism (\(Δ0–120\) min −1.56 ± 0.06 mm; \(P < 0.05\) versus euthyroid control) and even enhanced in hyperthyroidism (\(Δ0–120\) min −2.44 ± 0.13 mm; \(P < 0.01\) versus euthyroid control). Alterations in blood glucose resulted from an immediate fall in \(R_a\) in all thyroid states, whereas \(R_a\) was almost unaffected by the amount of insulin infused (Fig. 1). The initial fall in \(R_a\) varied with the thyroid state, being less pronounced in hypothyroidism (\(Δ0–30\) min −6.14 ± 0.66 μmol/min per kg; \(P < 0.01\) versus euthyroid control) and enhanced in hyperthyroidism (\(Δ0–30\) min −6.14 ± 0.66 μmol/min per kg; \(P < 0.01\) versus euthyroid control) compared with euthyroidism (\(Δ0–30\) min 3.75 ± 0.54 μmol/min per kg). The percentage decrease in \(R_a\) was 25, 30 and 38% in hypo-, eu- and hyper-thyroidism respectively. The rebound of increment in \(R_a\) started between 30 and 45 min of the infusion period (from 120 to 300 min, \(R_a\) remained decreased at 25, 20 and 22% of the initial value in eu-, hypo- and hyper-thyroid pigs respectively (Fig. 1).

Infusing insulin increased arterial lactate and pyruvate concentrations in all thyroid states (each \(P < 0.01\) (Fig. 2). Plasma glycerol decreased significantly after insulin infusion (\(P < 0.01\) in all thyroid states); the amount of decrease was most pronounced in hyper-thyroidism (\(Δ0–90\) min −60 ± 10 nmol/ml; \(P < 0.01\) versus euthyroid control) and less marked in hypo-thyroidism (\(Δ0–90\) min −18 ± 10 nmol/ml; \(P < 0.01\) versus euthyroid control). The basal glycerol values differed significantly between hypo- and eu-thyroid animals (\(P < 0.01\)), the decrease in serum glycerol in euthyroid controls (\(Δ0–90\) min) being −27 ± 9 nmol/ml (\(P < 0.01\)) (Fig. 2). Plasma alanine decreased after insulin infusion independently of the thyroid state (\(P < 0.05\) for all thyroid states) (Fig. 2). With respect to the post-infusion period, a rebound was observed for all parameters measured, which seems independent of the thyroid state (Fig. 2).

DISCUSSION

The essential finding of the present study is that, at physiological serum insulin concentrations, hyperthyroidism further increases the hypoglycaemic action of the hormone, whereas hypothyroidism decreases the response (Fig. 1). These data are contrary to those of other authors (Bratusch-Marrain et al., 1984; Dimitriadis et al., 1985; Laville et al., 1984). The observed discrepancies may be due in part to the experimental conditions applied: (i) as thyroid hormone action is dose-dependent, application of pharmacological amounts of \(T_3\) in the studies cited above may be misleading; (ii) the clinical severity of hyperthyroidism is variable; and (iii) drugs used in the treatment of the disease may interfere with glucose metabolism. However, our data support the idea of a further accelerated glucose turnover in hyperthyroidism (cf.
Saunders et al., 1980; McCulloch et al., 1983a; Sandler et al., 1983; Müller et al., 1983b), which may (at least partly) be due to the thyroid-hormone-induced increased sensitivity to other glucose-regulatory hormones. On the other hand, thyroidectomy decreases $R_i$ (McCulloch et al., 1983a) or is without effects on glucose turnover (Saunders et al., 1980; Müller et al., 1983b). From the above and from the finding that the insulin-provoked alterations in arterial glucogenic precursor supply were similar in all thyroid states (Fig. 2, except glycerol values), it is tempting to speculate (i) that both hormones act independently on glucose production and (ii) that hyperthyroidism may sensitize the hepatic response to insulin. From our data it is evident that moderate hyperthyroidism does not induce hepatic insulin resistance.

With respect to the peripheral action of insulin, it is well known from dose-dependence characteristics in vivo that insulin (at an arterial concentration of about 40 munits/ml) does not significantly affect total body glucose utilization (Rizza et al., 1981). On the other hand, insulin-induced inhibition of lipolysis occurs at lower serum insulin concentrations compared with the hypoglycaemic action of the hormone (Cheng & Kalant, 1970). Comparing plasma glycerol response to insulin in different thyroid states (Fig. 1), it is evident that the known anti-lipolytic action of insulin is even enhanced in hyperthyroidism and decreased in hypothyroidism (Fig. 1). Thus it is evident from the present data that, as in liver, no adipose-tissue resistance to insulin action is seen in hyperthyroidism. This is contrary to the data obtained in vitro by Wennlund et al. (1981), showing a decreased anti-lipolytic effect of insulin, in adipocytes from hyperthyroid patients. Even more confusing, the same authors (Arner et al., 1984) more recently reported an increase in insulin-induced glucose oxidation in hyperthyroid fat-cells; thus they speculate from their data in vitro that thyroid hormones selectively antagonize the anti-lipolytic effect of the hormones with a concomitant enhancement of the potency of insulin to increase cellular glucose metabolism. With respect to hypothyroidism, our data obtained in vivo correlate with the finding in vitro of a defective insulin
Hepatic insulin response in hypo- and hyper-thyroidism

action in hypothyroid rat adipocytes and soleus muscle (Czech et al., 1980).

To summarize, our data in vivo clearly demonstrate that moderate experimental hyperthyroidism does not antagonize hepatic or peripheral (adipose-tissue) insulin action, whereas hypothyroidism slightly diminished the insulin action.

We thank Mrs. A. Harneit and Mrs. D. Luda for expert technical assistance, Mrs. A. Smigilski for expert typing of the manuscript, Dr. P. Dimigen for veterinary supervision and Privat-Dozent Dr. W. Rehpenning for statistical advice. This work was supported by Sonderforschungsbereich 232.

REFERENCES


Received 31 May 1985/4 October 1985; accepted 8 November 1985

Vol. 234