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

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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 μ units/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 homeostasis. 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 μ units 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 T_3 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 Relliehausen, 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 ^{131}I /pig) 6–7 weeks before starting the experiments, leading to non-measurable serum T_4 and T_3 concentrations at least 4 weeks after treatment. Experimental hyperthyroidism resulted from a daily T_4 (Sigma) treatment for at least 10 days (5.0 μ g/kg body wt.), leading to elevated serum T_4 and T_3 values ($T_4 > 130$ ng/ml, $T_3 > 1.8$ ng/ml) compared with euthyroid animals ($T_4 = 36 \pm 3$ ng/ml, $T_3 = 1.3 \pm 0.1$ 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 μ mol of

Abbreviations used: R_t , rate of glucose turnover; R_a , rate of glucose production; R_d , rate of glucose utilization; T_4 , thyroxine; T_3 , L-3,3',5-tri-iodothyronine.

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 ($20 \mu\text{Ci}$) continuous ($0.20 \mu\text{Ci/min}$) infusion of [$3\text{-}^3\text{H}$]glucose (1 mCi/1 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.*

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

Data are given as means $\pm$ 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 R_a (eu- and hypo-thyroid $P < 0.05$; hyperthyroid not significant); alteration in R_d (not significant in all thyroid states).

(1983a, 1984), briefly: 0.5 ml blood samples were deproteinized by adding 1 ml of 5.35% (w/v) Ba(OH)₂, 1 ml of 5% (w/v) ZnSO₄·7H₂O 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 ³H 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_t) was calculated by the isotope-dilution equation: $R_t = F/SA$ (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

$$R_a = \{F - [pVc_t(dSA/dt)]\}/SA_t$$

and

$$R_d = pV(dc/dt),$$

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 pragmatical 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 ($\Delta 0$ –120 min -1.77 ± 0.22 mM), the effect was less pronounced in hypothyroidism ($\Delta 0$ –120 min -1.56 ± 0.06 mM; $P < 0.05$ versus euthyroid control) and even enhanced in hyperthyroidism ($\Delta 0$ –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_d 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 ($\Delta 0$ –30 min -6.14 ± 0.66 μ mol/min per kg; $P < 0.01$ versus euthyroid control) and enhanced in hyperthyroidism ($\Delta 0$ –30 min -6.14 ± 0.66 μ mol/min per kg; $P < 0.01$ versus euthyroid control) compared with euthyroidism ($\Delta 0$ –30 min 3.75 ± 0.54 μ mol/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 hyperthyroidism ($\Delta 0$ –90 min -60 ± 10 nmol/ml; $P < 0.01$ versus euthyroid control) and less marked in hypothyroidism ($\Delta 0$ –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 ($\Delta 0$ –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 T₃ 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.

Fig. 2. Effect of continuous infusion of insulin (0.4 munit/min per kg for 6 h) on arterial plasma lactate, pyruvate, glycerol and alanine in conscious unrestrained 24 h-starved hypo-, eu- and hyper-thyroid miniature pigs

Data are given as means $\pm$ S.E.M. ($n = 5$). Statistical significance for the alterations induced by the insulin infusion: increase in lactate ($P < 0.01$) in all thyroid states, decrease in glycerol and alanine ($P < 0.01$) in all thyroid states.

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_t (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 μ units/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

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.

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REFERENCES

- Arner, P., Bolinder, J., Wennlund, A. & Östman, J. (1984) *Diabetes* **33**, 369–375
- Bratusch-Marrain, P. R. (1983) *Diabetologia* **24**, 74–79
- Bratusch-Marrain, P. R., Gasic, S. & Waldhäusl, W. K. (1984) *Am. J. Physiol.* **247**, E681–E687
- Cheng, J. S. & Kalant, F. (1970) *J. Clin. Endocrinol. Metab.* **31**, 647–653
- Cowan, J. S. & Hetenyi, G., Jr. (1971) *Metab. Clin. Exp.* **20**, 360–372
- Czech, M. P., Malbon, C. G., Kerman, K., Gitomer, G. & Pilch, P. F. (1980) *J. Clin. Invest.* **66**, 574–582
- Dimitriadis, G., Baker, B., Marsh, H., Mandarino, L., Rizza, R., Bergman, R., Haymond, M. & Gerich, J. (1985) *Am. J. Physiol.* **248**, E593–E601
- Laville, M., Riou, J. P., Bougneres, P. F., Canivet, B., Beylot, M., Cohen, R., Serusalat, P., Dumontet, C., Berthazene, F. & Mornex, R. (1984) *J. Clin. Endocrinol. Metab.* **58**, 960–965
- Lenzen, S. & Bailey, L. J. (1984) *Endocr. Rev.* **5**, 411–434
- McCulloch, A. J., Nosadini, R., Pernet, A., Piniewska, M., Cook, D. B., Clark, F., Johnston, D. G. & Alberti, K. G. M. M. (1983a) *Clin. Sci.* **64**, 41–47
- McCulloch, A. J., Home, P. D., Heine, R., Ponchner, M., Hanning, I., Johnston, D. G., Clark, F. & Alberti, K. G. M. M. (1983b) *Clin. Endocrinol. (Oxford)* **18**, 327–334
- Müller, M. J. & Seitz, H. J. (1984) *Klin. Wochenschr.* **62**, 11–18, 49–55, 97–102
- Müller, M. J., Paschen, U. & Seitz, H. J. (1983a) *Am. J. Physiol.* **244**, E236–E244
- Müller, M. J., Paschen, U. & Seitz, H. J. (1983b) *Endocrinology (Baltimore)* **112**, 2025–2031
- Müller, M. J., Paschen, U. & Seitz, H. J. (1984) *J. Clin. Invest.* **74**, 249–261
- Paschen, U., Müller, M. J. & Seitz, H. J. (1982) *J. Surg. Res.* **32**, 586–597
- Rizza, R. A., Mandarino, L. J. & Gerich, J. E. (1981) *Am. J. Physiol.* **240**, E630–E639
- Sandler, M. P., Robinson, R. P., Rabin, D., Lacy, W. W. & Abumrad, N. N. (1983) *J. Clin. Endocrinol. Metab.* **56**, 479–485
- Saunders, J. S., Hall, E. H. & Sönksen, P. H. (1980) *Clin. Endocrinol. (Oxford)* **13**, 33–44
- Shen, D.-C. & Davidson, M. B. (1985) *J. Clin. Endocrinol. Metab.* **60**, 1038–1041
- Steele, R. (1959) *Ann. N.Y. Acad. Sci.* **82**, 420–430
- Wennlund, A., Arner, P. & Östman, J. (1981) *J. Clin. Endocrinol. Metab.* **53**, 631–635
- Wolfe, R. R. (1984) *Tracers in Metabolic Research*, A. R. Liss, New York
- Yasuda, H., Harano, Y., Ohgaku, S., Kosugi, K., Suzuki, M., Hidaka, H., Kashiwagi, A. & Shigeta, Y. (1984) *Horm. Metab. Res.* **16**, 3–6

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