fasting plasma glucose levels were determined the next morning. Mice were then injected i.p. with 2 g/kg of dextrose (Abbott Labs, North Chicago, IL) and plasma glucose levels were determined 1 and 2 h later. Mice were considered to demonstrate abnormal glucose tolerance when at least two of three plasma glucose values (i.e., 0, 1, and 2 h) exceeded the respective mean values obtained from control animals by greater than 3 standard deviations. In all instances, blood samples were collected using heparinized capillary tubes via the retro-orbital plexus. Plasma glucose concentrations were analyzed by the glucose-oxidase method using a glucose analyzer (Beckman Instruments, Fullerton, CA).

Statistical analyses were by chi-square test using the Yates' correction. Probability values of 0.05 or less were considered significant.

All animals were sacrificed after the last plasma glucose determination and the pancreata were fixed in Bouin's solution and processed for light microscopy. Sections were stained with hematoxylin and eosin or aldehyde fuchsin and were examined. The histologic appearance of islets was consistent with plasma glucose levels. Severely diabetic mice, regardless of type of diet, had islets with degranulated and necrotic beta cells while islets from non-diabetic mice had minimal or less severe damage.

*Results and discussion.* The table shows the mean plasma glucose values for deficient and control mice 1, 2, and 5 days after STZ injection. Untreated mice on the control and deficient diet had nearly identical mean plasma glucose values. However, the incidence of diabetes on both days 2 ($p < 0.001$) and 5 ($p < 0.05$) was higher in STZ-treated mice receiving the deficient diet than in those receiving the control diet. At every dose, the degree of hyperglycemia (i.e., mean plasma glucose level) in the diabetic mice on the deficient diet exceeded that of the mice on the control diet. Furthermore, abnormal glucose tolerance was more frequent in the deficient group. These data demonstrate that niacin/nicotinamide deficient CD-1 mice show a markedly enhanced susceptibility to the diabetogenic effect of STZ. Both STZ[3-10, 14, 15] and dietary niacin deficiency[17] are known to decrease NAD levels in islets. If STZ induces beta cell necrosis and diabetes by critically depleting the beta cells of NAD, it is not surprising that these two treatments have an additive or synergistic effect. Finally, niacin/nicotinamide deficiency may also lower STZ's LD-50. Three of five mice on the deficient diet receiving 160 mg/kg died; the LD-50 for mice on the control diet exceeded 200 mg/kg (data not shown).

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## Thermogenic effects of thyrotrophin-releasing hormone and its analogues in the rat

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*Summary.* Acute or chronic injection of RX 77 368 (a TRH analogue; 1 mg/kg s.c.) stimulated oxygen consumption ($VO_2$) and brown adipose tissue activity in the rat, and decreased weight gain. Other TRH analogues (CG 3509, RGH 2202) and TRH itself also stimulated $VO_2$. These thermogenic actions are probably mediated centrally by stimulation of sympathetic outflow to brown fat.
*Key words.* TRH; TRH analogues; thermogenesis; brown adipose tissue.

Thyrotrophin releasing hormone (TRH) was first identified as a hypothalamic hormone releasing thyroid stimulating hormone (TSH) from the pituitary gland[1]. In common with many other peptide hormones, TRH has additionally been ascribed a role as a central neurotransmitter or neuromodulator with a variety of physiological actions[1, 2]. For example, TRH affects thermoregulation in many species[3-7], although the responses may be somewhat varied. Peripheral administration of TRH usually induces hyperthermia[6, 7], but this may be at least partly dependent on release of TSH and a subsequent rise in circulating thyroid hormone levels[7, 8]. Central administration of TRH can also induce hyperthermia and reverse experimentally-induced hypothermia[9, 10], but in some studies a reduction in body temperature has been observed[9, 11].

Increases in body temperature can be achieved either by reductions in heat loss or by stimulation of heat production. In small mammals, non-shivering thermogenesis which al-

lows animals to maintain homeothermy during cold-exposure, is largely dependent on sympathetic activation of brown adipose tissue (BAT)[12–14]. Thus, it seems possible that the hyperthermic actions of TRH might involve activation of the sympathetic nervous system and brown fat. Surprisingly, Sato et al.[9] reported a fall in BAT activity in immature rats treated with TRH centrally, but concluded that while TRH itself stimulates thermogenesis, its metabolite cyclo(histidine-proline) inhibited heat production. This, together with the very short duration of action of TRH, poses quite serious experimental problems in the study of TRH and thermoregulation. However, these may be partly overcome by the use of more stable analogues of TRH which are now available[2]. These analogues not only offer the advantage of considerably extended biological half-life, but also show some selectivity in their central and peripheral actions. We have taken advantage of these TRH analogues to investigate their effects on metabolic rate and BAT activity in the rat.

*Materials and methods.* Studies were performed on young (6–8 weeks) male, Sprague-Dawley rats (Charles River, Kent, U.K.), allowed ad libitum access to pelleted stock diet. Hypophysectomy (HYPX) was performed on some animals by suction of the pituitary through a trefine hole in the skull under halothane anaesthesia, and subsequently verified from the change in testiclar and adrenal weights.

Resting oxygen consumption ($VO_2$) was measured in closed-circuit respirometers[15] at 29 °C, for 2 h before and up to 3 h after injection of either RX 77 368 (pGlu-His-(3,3 dimethyl) $ProNH_2$; 0.5 or 1.0 mg/kg s.c.; gift from Dr P. Dettimar, Reckitt & Colman, Hull), CG 3509 (orotyl-His-$ProNH_2$; 0.5 or 1.0 mg/kg s.c.; gift from Dr L. Floke, Chemie Grunenthal, Aachen, FRG), TRH (pGlu-His-$ProNH_2$; 0.3 mg/kg s.c.; Bachem U.K., Essex), or RGH 2202 (L-pyro-2-aminoadipyl-Leu-$ProNH_2$; 0.5 mg/kg s.c.; gift from Dr T. Szirtes, Gideon Richter & Co. Ltd, Budapest, Hungary). In some cases, animals were pretreated with the $\beta$-adrenergic antagonist propranolol (20 mg/kg s.c.). The acute effect of RX 77 368 on BAT activity was tested by removing interscapular BAT from rats killed 1 h after injection of this compound (1 mg/kg s.c.) or vehicle alone. The activity of the BAT mitochondrial proton conductance pathway was assessed from the binding of $^3$H-guanosine diphosphate (GDP, Amersham International, Bucks, U.K.) to mitochondria isolated by differential centrifugation[16].

Separate groups of rats were injected with either RX 77 368 (1 mg/kg) or saline, daily for 10 days. This analogue was chosen for the chronic trial because it was the most stable and exerts the greatest central effects[2]. Food intake and body weight were recorded daily, and metabolisable energy (ME) intake was calculated from the ME density of the diet. Resting $VO_2$ was measured on day 7, before and after injection of noradrenaline (0.25 mg/kg s.c.) in order to test maximal thermogenic capacity (no injection of RX 77 368 was given on this day). The animals were killed on day 10, and the mass and protein content (dye-reagent method, BioRad, Watford, U.K.) of the interscapular BAT depot was determined. The activity of the proton conductance pathway was assessed from the binding of $^3$[H]GDP to isolated mitochondria.

Values are presented as means ± SEM. Statistical differences were assessed by the Student's t-test for unmatched data.

*Results.* The data for $VO_2$ presented in table 1 indicate that the TRH analogues, RX 77 368 and CG 3509, both caused marked increases in metabolic rate in normal rats (peak values at 70–95 min) without any apparent effect on physical activity. These responses were dose-dependent, and the effect of RX 77 368 was inhibited (by 53%) but not prevented by prior treatment with propranolol. In hypophysectomized rats, CG 3509 and RX 77 368 caused significant increases in metabolic rate but these were attenuated compared to intact animals. A single injection of RX 77 368 caused a 70% increase ($p < 0.05$) in GDP-binding (control 48 ± 3, RX 77 368 82 ± 9 pmol/mg mitochondrial protein; mean ± SEM, n = 6). TRH and RGH 2202 both produced increases in metabolic rate of about 20% at a dose of 0.5 mg/kg (table 1).

Animals treated chronically with RX 77 368 (table 2) showed a reduction in body weight gain and the efficiency of gain (gain per unit intake), but food intake was similar to controls. Resting $VO_2$ was comparable for control rats and those injected with RX 77 368, but the response to injection of noradrenaline was enhanced by 31%. Interscapular BAT mass and protein content were unaffected by chronic injection of RX 77 368, but BAT activity (GDP-binding) was elevated by 80% at the end of the experiment.

*Discussion.* Peripheral injection of a relatively low dose (0.5 mg/kg b. wt) of TRH or the three TRH analogues all caused comparable increases in oxygen consumption of 20–

Table 1. Resting oxygen consumption (ml/min/kg$^{0.75}$) before and after injection of TRH and analogues

| | Dose (mg/kg) | Before | After | Increment | Increase % | n |
|---|---|---|---|---|---|---|
| *RX 77 368* | | | | | | |
| Control | 0.5 | 13.5 ± 0.4 | 15.9 ± 0.4 | 2.4 ± 0.1 | 18 ± 1 | 4 |
| | 1.0 | 13.1 ± 0.5 | 18.4 ± 0.9 | 5.2 ± 0.1 | 40 ± 3 | 9 |
| Propanolol | 1.0 | 11.5 ± 0.6 | 14.0 ± 0.6 | 2.5 ± 0.1[b] | 22 ± 2[a] | 5 |
| HYPX | 1.0 | 13.2 ± 0.4 | 17.3 ± 1.0 | 4.1 ± 1.0 | 31 ± 4 | 4 |
| *CG 3509* | | | | | | |
| Control | 0.5 | 13.9 ± 0.6 | 18.1 ± 0.5 | 4.2 ± 0.3 | 30 ± 4 | 4 |
| | 1.0 | 13.9 ± 0.1 | 20.2 ± 0.6 | 6.4 ± 0.6 | 46 ± 4 | 6 |
| HYPX | 1.0 | 13.1 ± 0.3 | 16.7 ± 0.7 | 3.6 ± 0.5 | 27 ± 3 | 6 |
| *TRH* | | | | | | |
| Control | 0.5 | 11.9 ± 0.2 | 14.7 ± 0.4 | 2.8 ± 0.3 | 23 ± 2 | 5 |
| *RGH* | | | | | | |
| Control | 0.5 | 12.4 ± 0.3 | 14.6 ± 0.4 | 2.3 ± 0.4 | 19 ± 2 | 5 |

Resting oxygen consumption measured for 2 h before and up to 3 h after treatment. Values are means ± SEM with TRH analogues. Propanolol (20 mg/kg) given at time zero; hypophysectomy (HYPX) performed at least 5 days previously. $VO_2$ after a single injection of analogues was significantly greater ($p < 0.05$) than $VO_2$ before injection in all cases. [a] $p < 0.01$, [b] $p < 0.001$ compared to control rats given the same dose of drug.

Table 2. Chronic effects of RX 77 368

| | | |
|---|---|---|
| Final body weight (g) | 194 ± 5 | 184 ± 3 |
| Body weight gain (g) | 76 ± 3 | 65 ± 2[a] |
| Metabolisable energy intake (kJ) | 2600 ± 20 | 2595 ± 40 |
| Efficiency of weight gain (g gain/MJ eaten) | 29 ± 1 | 25 ± 1 |
| Resting $VO_2$ (ml/min/kg$^{0.75}$) | | |
| Before noradrenaline | 15.7 ± 0.3 | 15.7 ± 0.4 |
| After noradrenaline | 22.9 ± 0.4 | 25.4 ± 0.8[a] |
| Increment | 7.2 ± 0.4 | 9.4 ± 0.8[a] |
| % Increase | 46 ± 2 | 60 ± 4[a] |
| Interscapular BAT | | |
| Mass (mg) | 198 ± 14 | 193 ± 14 |
| Protein content (mg) | 17 ± 1 | 60 ± 4 |
| Specific mitochondrial GDP binding (pmol/mg protein) | 51 ± 2 | 92 ± 5[b] |

Rats were treated with saline or RX 77 368 (1 mg/kg) daily for 10 days. Dose of noradrenaline was 250 µg/kg, s.c. Mean values ± SEM (n = 8). [a] $p < 0.05$, [b] $p < 0.001$ compared to control.

30%. Increases of approximately 50% were obtained with higher doses of RX 77 368 and CG 3509, but in a small number of animals (n = 3–4) we have found no further increases in response to 2.5 mg/kg of these compounds (data not shown). In a separate study we observed that a higher dose of TRH (1.0 mg/kg) elicited increases in $VO_2$ of 35% (Rothwell and Stock, unpublished data). The results indicate that the effects of TRH and its analogues were due largely to a central action, and this probably does not involve release of TSH, and hence may be unrelated to circulating thyroid hormone levels. Hypophysectomy caused slight inhibition of the effects of CG 3509 and RX 77 368 on $VO_2$, but a significant increase in metabolic rate was still observed in these animals. Inhibition of the sympathetic nervous system by injection of propranolol caused a marked reduction in $VO_2$ after injection of RX 77 368, but did not completely abolish the response. The observation that RGH 2202 stimulated $VO_2$ to a similar extent as other TRH analogues further supports the suggestion that these effects are mediated centrally, since this analogue is claimed to have very low endocrine activity and to exert most of its actions centrally[17]. Sympathetically-mediated thermogenesis is largely dependent on activation of heat production in brown adipose tissue via the mitochondrial proton conductance pathway[12–14] and injection of RX 77 368 caused marked stimulation of this pathway (i.e. GDP-binding). These results indicate that the thermogenic effects of TRH analogues may be due to increased sympathetic activation of heat production in brown fat. Some of the TRH analogues studied will release TSH and possibly triiodothyronine and thyroxine, both of which stimulate metabolic rate, but the time course of these effects is very different (12–14 h) to those observed, and these hormones do not stimulate BAT[18].

Chronic injection of RX 77 368 decreased body weight gain in animals maintaining a normal level of food intake, suggesting that stimulation of metabolic rate by this compound was sustained throughout the study. No difference in $VO_2$ was observed 24 h after injection probably because of the relatively short duration of action of this drug. The effect of noradrenaline injection on metabolic rate is routinely used to assess thermogenic capacity and is largely due to stimulation of heat production in brown adipose tissue[12–14]. The response to noradrenaline and the activity of the proton conductance pathway in brown fat (assessed from GDP-binding) were both increased following chronic treatment with TRH.

The acute and chronic stimulation of thermogenesis by TRH analogues observed in this study have two important implications. Firstly, they suggest that TRH itself may have a physiological role in the control of thermogenesis, probably via central actions. Secondly, these effects may be relevant to the development of TRH analogues for clinical purposes and suggest a further possible use of these compounds in the control of energy metabolism and body weight.

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