Central effects of thyronamines on glucose metabolism in rats

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Abstract

Thyronamines are naturally occurring, chemical relatives of thyroid hormone. Systemic administration of synthetic 3-iodothyronamine (T1AM) and – to a lesser extent – thyronamine (T0AM), leads to acute bradycardia, hypothermia, decreased metabolic rate, and hyperglycemia. This profile led us to hypothesize that the central nervous system is among the principal targets of thyronamines. We investigated whether a low dose i.c.v. infusion of synthetic thyronamines recapitulates the changes in glucose metabolism that occur following i.p. thyronamine administration. Plasma glucose, glucoregulatory hormones, and endogenous glucose production (EGP) using stable isotope dilution were monitored in rats before and 120 min after an i.p. (50 mg/kg) or i.c.v. (0.5 mg/kg) bolus infusion of T1AM, T0AM, or vehicle. To identify the peripheral effects of centrally administered thyronamines, drug-naïve rats were also infused intravenously with low dose (0.5 mg/kg) thyronamines. Systemic T1AM rapidly increased EGP and plasma glucose, increased plasma glucagon, and corticosterone, but failed to change plasma insulin. Compared with i.p.-administered T1AM, a 100-fold lower dose administered centrally induced a more pronounced acute EGP increase and hyperglucagonemia while plasma insulin tended to decrease. Both systemic and central infusions of T0AM caused smaller increases in EGP, plasma glucose, and glucagon compared with T1AM. Neither T1AM nor T0AM influenced any of these parameters upon low dose i.v. administration. We conclude that central administration of low-dose thyronamines suffices to induce the acute alterations in glucoregulatory hormones and glucose metabolism following systemic thyronamine infusion. Our data indicate that thyronamines can act centrally to modulate glucose metabolism.

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Introduction

Thyronamines are a group of naturally occurring, chemical relatives of thyroid hormone (TH) with pronounced and rapid physiologic effects (Scanlan et al. 2004). Two representatives of the thyronamines, 3-iodothyronamine (T1AM) and thyronamine (T0AM), have been extracted from rat and mouse brain, heart, liver, and blood. T1AM and T0AM can theoretically be derived from iodothyronines thyroxine (T4), 3,3’,5-triiodothyronine (T3), and/or 3,3’, 5’-triiodothyronine (reverse T3) by removal of the carboxylate group on the β-alanine side chain in addition to deiodination. Indeed, thyronamines have recently been identified as isoenzyme-specific substrates of the iodothyronine deiodinases type 1, 2, and 3 (Piehl et al. 2008). T1AM and, to a lesser extent, T0AM are potent in vitro agonists of the trace amine-associated receptor type 1 (TAAR1; Scanlan et al. 2004, Hart et al. 2006), a Gs protein–coupled membrane receptor with a broad expression profile (Grandy 2007). In rodents and humans, high levels of TAAR1 expression are found in liver, kidney, gastrointestinal tract, pancreas, heart, and many areas of the brain (Borowsky et al. 2001, Bunzow et al. 2001). Moreover, T1AM has the potential to act as an adrenergic receptor α2 (ARα2) agonist in the mouse, explaining in part the decrease in insulin secretion by pancreatic β-cells exposed to thyronamines (Regard et al. 2007).

When administered to rodents, T1AM and T0AM have striking effects on physiology. Within minutes after systemic administration, profound hypothermia, bradycardia, and decreased cardiac output occur. In addition, thyronamines rapidly induce metabolic alterations such as decreased metabolic rate and a dramatic shift to preferential lipid fuelling at the cost of carbohydrate oxidation (Scanlan et al. 2004, Braulke et al. 2007). These apparently non-genomic effects are thought to occur via binding to and activating membrane-bound G protein–coupled receptors (GPCRs) such as TAAR1 and ARα2 (Liggett 2004, Scanlan et al. 2004). Furthermore, it has been proposed but not yet demonstrated that THs can be converted to thyronamines by enzymatic deiodination and decarboxylation. Since most
actions of T\(_1\)AM and T\(_0\)AM are opposite in direction to the bioactive TH T\(_3\), thyronamines have been hypothesized to play a role in fine-tuning and/or antagonizing T\(_3\) actions on a moment-to-moment timescale (Liggett 2004, Weatherman 2007).

The brain, in particular the hypothalamus, regulates most of the processes affected by thyronamines (body temperature, cardiac function, and energy metabolism). Moreover, a principal role in regulating hepatic glucose metabolism has recently emerged for the hypothalamus (Obici et al. 2002, Kalsbeek et al. 2004, Pocai et al. 2005). As T\(_1\)AM and T\(_0\)AM are present in rat brain, we hypothesized that these novel compounds could affect glucose metabolism via actions in the central nervous system (CNS).

In the present study, we tested the hypothesis that thyronamines act centrally to induce changes in glucose metabolism using stable isotope dilution and three different routes of administration: systemic (i.p.), central (i.c.v.), and i.v. in rats. Our results are consistent with the interpretation that T\(_1\)AM and T\(_0\)AM can act centrally to recapitulate the changes in glucose metabolism that occur following systemic thyronamine administration.

### Materials and Methods

**Animals**

Male Wistar rats (Harlan, Horst, The Netherlands) between 350 and 400 g body weight (BW), housed under constant conditions of temperature (21 ± 1°C) and humidity (60 ± 2%) with a 12 h light:12 h darkness schedule (lights on at 0700 h), were used in all experiments. Food and drinking water were available ad libitum. All of the following experiments were conducted with the approval of the Animal Care Committee of the Royal Netherlands Academy of Arts and Sciences.

**T\(_1\)AM and T\(_0\)AM**

T\(_1\)AM-HCl (391 g/mol) and T\(_0\)AM-HCl (264 g/mol) were synthesized as previously reported (Hart et al. 2006) and dissolved in 20% DMSO and 80% saline (vehicle) at a concentration of 40 mg/ml.

**Experimental groups**

Two independent studies were performed. For the first study, permanent jugular vein and carotid artery cannulae were placed in rats (n = 22) under anesthesia (see below). Animals were allowed to recover from the surgery for 8 days prior to any further manipulations. Each rat thus cannulated received an i.p. bolus infusion of 50 mg/kg of T\(_1\)AM (n = 7), 50 mg/kg of T\(_0\)AM (n = 8), or an equal volume (500 µl) of vehicle (n = 7). For the second study, rats (n = 31) were equipped with a guide cannula placed into the left lateral cerebral ventricle in addition to the carotid artery and jugular vein cannulae. Rats thus cannulated received an i.c.v. 100-fold lower dose (0.5 mg/kg) of either T\(_1\)AM (n = 9), T\(_0\)AM (n = 8), or DMSO-saline vehicle (n = 8) in a volume of 4 µl. To control for the possibility that any observed effect of the i.c.v.-infused thyronamines was somehow due to spillover into the circulation, an additional group of cannulated rats was infused intravenously with 0.5 mg/kg T\(_1\)AM (n = 3) and T\(_0\)AM (n = 3) in a volume of 500 µl. In both of these experiments, before and 120 min after i.p. or i.c.v. bolus infusion, isotope dilution and blood sampling were conducted to permit measurement of endogenous glucose production (EGP), and the concentration of plasma glucose, insulin, glucagon, corticosterone, thyroid stimulating hormone (TSH), T\(_3\) and T\(_4\) concentrations.

**Surgery**

Animals were anaesthetized using a mixture of Hypnorm (Janssen; 0.05 ml/100 g BW, i.m.) and Dormicum (Roche; 0.04 ml/100 g BW, s.c.). Vascular and i.c.v. cannulae were fixed with dental cement to four stainless-steel screws inserted into the skull. Post-operative care was provided by s.c. injection of 0.01 ml/100 g BW of Temgesic (Schering-Plough, Utrecht, The Netherlands). In all animals, an intra-atrial silicone cannula was implanted through the right jugular vein and a second silicone cannula was placed in the left carotid artery for isotope infusion and blood sampling as described previously (Klieverik et al. 2008). For the second study, stainless-steel i.c.v. cannulae were implanted into the left cerebral ventricle using the following stereotaxic coordinates: anteroposterior: −0.8 mm; lateral: ±2.0 mm; ventral: −3.2 mm, with the toothbar set at −3.4 mm. Guide cannula placement was confirmed by dye (4 µl of ethylene blue) injection and inspection post-mortem. Only animals that showed staining of the left lateral cerebral ventricle and third cerebral ventricle were included in the final analysis.

**Stable isotope dilution and systemic versus central thyronamine administration**

Eight days post-surgery, stable isotope dilution was performed in combination with the administration of synthetic thyronamines. Animals weighed between 335 and 380 g. BW increased in all groups during the 3 days preceding the experimental infusions, indicating recovery from surgery and a positive energy balance. One day before the experimental infusions, rats were connected to a metal collar to which polyethylene tubing (for blood sampling and infusion) was attached and kept out of reach of the animals by a counterbalanced beam. This permitted all subsequent manipulations to be performed outside the cages without handling the animals (Klieverik et al. 2008). For determining basal plasma concentrations of TSH, T\(_3\), and T\(_4\), a blood sample was obtained at 1400 h. On the day...
of thyronamine administration, food was removed from the cages 4 h (≈0830 h) before the first basal measurements. At ≈1100 h, a blood sample was taken (200 μl, t = −110 min) for determination of background isotopic enrichment. Subsequently, a primed (8-0 μmol in 5 min) continuous (16-6 μmol/h) infusion of the stable isotope tracer [6,6-2H2]-glucose (>99% enriched; Cambridge Isotope Laboratories, Cambridge, MA, USA) was started using an infusion pump (Harvard Apparatus, Holliston, MA, USA). After an equilibration period of 90 min, additional blood samples (200 μl) were obtained for the determination of basal plasma glucose, isotopic enrichment (t = −20, −10 and 0 min), plasma corticosterone (t = −20 and 0 min), insulin, and glucagon (t = 0 min) concentrations.

After the t = 0 blood sample, in study 1, animals received an i.p. bolus of either T1AM, T0AM (50 mg/kg in 500 μl), or vehicle. In study 2, again after the t = 0 blood sample, animals received an i.c.v. bolus infusion of either T1AM, T0AM (0·5 mg/kg in 4 μl), or vehicle delivered through the i.c.v. cannula in 105 s using a Hamilton syringe. After i.p. or i.c.v. bolus infusion, blood samples were obtained for measurement of glucose concentration, isotopic enrichment (5, 10, 20, 30, 45, 60, 75, 90, and 120 min), plasma corticosterone (t = 10, 20, 30, 60, and 120 min), plasma insulin, glucagon (t = 10, 60 and 120 min), and plasma TSH, T3, and T4 concentrations (t = 120 min).

**Plasma hormone and isotope analyses**

Plasma glucose concentration was determined in triplicate by a glucose oxidase method (Boehringer Mannheim). Plasma glucagon and corticosterone were measured using a radioimmunoassay (Kalsbeek et al. 2000), with inter- and intra-variation coefficients (CV) of 7–8 and 3–4% (T3), and 3–6 and 2–4% (T4) respectively. Detection limits for T3 and T4 were 0·3 and 5 nmol/l respectively. Plasma TSH concentrations were determined by an in-house RIA (Kalsbeek et al. 2000), with detection limits for TSH of 4 and 2% at ±3·5 mU/l respectively with a detection limit of 0·40 mU/l. Plasma insulin was measured by a commercially available ELISA (Mercodia, Uppsala, Sweden; Ackermans et al. 2008). The inter- and intra-variation CVs for TSH were <4 and 2% at ±3·5 mU/l respectively with a detection limit of 0·40 mU/l. Plasma insulin was measured by a commercially available ELISA (Mercodia, Uppsala, Sweden; Ackermans et al. 2000). The [6,6-2H2]-glucose enrichment (tracer/tracee ratio) inter-assay CV was 1%, the intra-assay CV 1%, and the detection limit 0·04%.

**Calculations and statistical analysis**

EGP was calculated from isotope enrichment and plasma glucose concentration using modified forms of steady-state (basal) and non-steady-state (after thyronamine infusion) Steele equations (Steele 1959). Data were analyzed by two-way ANOVA with repeated measurements, with treatment group (T1AM, T0AM, or Veh) and time as dependent factors. Significance was defined at P<0·05 using paired t-tests (i.e. within treatment groups) and independent t-tests (i.e. between treatment groups) to identify experimental groups that differed significantly. The SPSS statistical software program version 16·0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Data are presented as mean ± S.E.M.

**Results**

In two independent studies, eight groups of rats were investigated. In the first study, rats received an i.p. bolus infusion of either T1AM, T0AM (0·5 mg/kg, n = 7), or vehicle (n = 7). In the second study, rats were intracerebroventricularly infused with a 100-fold lower dose (i.e. 0·5 mg/kg) of either T1AM (n = 9), T0AM (n = 8), or vehicle (n = 8). To address the possibility that any physiologic response observed following i.c.v. infusion of the thyronamines was due to the peripheral action of drug that spilled over into the circulation, two additional groups of animals were intravenously infused with 0·5 mg/kg of T1AM (n = 3) or 0·5 mg/kg of T0AM (n = 3).

**Study #1: systemic thyronamine infusion**

Rats injected i.p. with 50 mg/kg T1AM or 50 mg/kg T0AM exhibited a behavioral phenotype as described previously (Scanlan et al. 2004). Interestingly, the animals injected with T1AM displayed the more robust phenotype even though the dose (on a per mole basis) was approximately half that of T0AM.

**Glucose homeostasis: plasma concentration and endogenous production**

Systemic infusion of either T1AM or T0AM by the i.p. route of administration induced a rapid and significant increase in plasma glucose concentration (Fig. 1a). The onset and magnitude of this effect was similar for the two compounds until 45 min post-infusion when the effect of T0AM apparently plateaued, while the T1AM-induced hyperglycemia continued to develop eventually reaching a maximum 371 ± 27% of basal values 120 min after infusion.

Within 10 min of receiving an i.p. bolus of T1AM, the EGP increased to 143 ± 3% of basal values (P = 0·001 versus Veh; Fig. 1b), which was sustained for the duration of the experiment. Similarly, i.p. administration of T0AM rapidly increased EGP, reaching a maximum of 158 ± 16% of basal values after 20 min (P = 0·032 versus Veh). Forty-five minutes after injection with T0AM, EGP gradually returned to basal values by t = 120 min.
Glucoregulatory hormones Given the profound effect of T1AM and T0AM on plasma glucose and EGP, we characterized the status of three glucoregulatory hormones: insulin; glucagon; and corticosterone (Fig. 2). Surprisingly, even though T1AM and T0AM (50 mg/kg, i.p.) produced hyperglycemia and elevated EGP, plasma insulin concentrations were unchanged relative to plasma from vehicle-injected rats (Fig. 2a). By contrast, plasma glucagon concentrations were significantly increased within 10 min of either T1AM or T0AM administration (Fig. 2b). However, by 60 min post-injection, the time-effect profiles of the two compounds had begun to diverge with T0AM’s effect reaching a plateau at 240% (P = 0.007 T0AM versus Veh, t = 60) of basal levels, while T1AM’s effect continued to develop for the duration of the experiment (447 ± 44% of basal values at 120 min, P < 0.0001 T1AM versus Veh; Fig. 2b).

Plasma corticosterone displayed a significant increase in response to both T1AM- and T0AM-injected i.p., compared with vehicle-injected rats (Fig. 2c). T1AM infusion induced a maximal increase at t = 60 min (482 ± 106 vs 179 ± 71% of basal levels at t = 60 min, T1AM versus Veh; P = 0.022). T1AM infusion increased plasma corticosterone to a similar extent (393 ± 59 vs 172 ± 102% of basal levels at t = 120 min, T0AM versus Veh; P = 0.022). At no time point was there a difference in the corticosterone response between T1AM- and T0AM-infused groups.

Plasma T3, T4, and TSH concentrations before and 120 min after i.p. T1AM, T0AM, and vehicle infusion are depicted in Table 1. Within 120 min, both T4 and TSH levels were significantly decreased in response to i.p. T1AM or, to a larger extent, i.p. T0AM (50 mg/kg). Intriguingly, T3 levels were also decreased 120 min after i.p. T0AM (50 mg/kg) when compared with vehicle-injected rats.

Study #2: central thyronamine infusion I.c.v. infusion of 0.5 mg/kg T1AM or T0AM did not induce any of the phenotypical alterations observed after systemic thyronamine administration.

Glucose homeostasis: plasma concentration and endogenous production With an i.c.v. bolus infusion of 0.5 mg/kg, T1AM plasma glucose concentration began to increase immediately (Fig. 3a) until it reached a maximum 199 ± 13% of basal levels 30 min after infusion (P < 0.0001 versus Veh). During the next 90 min, plasma glucose decreased slightly stabilizing at ~163% of basal values. T0AM (0.5 mg/kg, i.c.v.) significantly elevated plasma glucose as well, but to a lesser degree than T1AM (maximum 134 ± 6% at t = 45, P < 0.0001 versus Veh; Fig. 3a).

T1AM (0.5 mg/kg, i.c.v.) induced a rapid and significant increase in EGP (Fig. 3b) by 10 min post-infusion reaching a maximum 178 ± 16% of basal values at 30 min (P < 0.0001 versus Veh), which gradually decreased with time to 113 ± 5% when the experiment was terminated at t = 120 min. Although T0AM (0.5 mg/kg, i.c.v.) significantly increased EGP above basal levels (P < 0.0001; t = 0 vs t = 10), its maximum effect (20 ± 9% increase at t = 20; P = 0.069 versus Veh) was approximately one-third of T1AM’s maximal effect (Fig. 3b).

Importantly, when T1AM or T0AM were infused intravenously at the dose that was used in the i.c.v. infusion experiments (0.5 mg/kg, T1AM n = 3, T0AM n = 3) neither thyronamine had any effect on plasma glucose concentrations, EGP, plasma insulin, nor glucagon at any time point when compared with basal values (data not shown).
When the absolute changes in plasma glucose concentration produced by 0.5 mg/kg T1AM i.c.v. and 50 mg/kg T1AM i.p. are compared over time, their profiles are practically superimposable for the first 30 min of exposure (Fig. 4a). Thereafter, they diverged as the systemic effect of T1AM continued to develop. Plotting the absolute values for EGP in response to 0.5 mg/kg T1AM i.c.v. and 50 mg/kg T1AM i.p. revealed both routes of administration-produced identical profiles during the initial 20 min post-exposure (Fig. 4b). However, at 30 min thereafter, the magnitude of the EGP effect elicited by T1AM i.c.v. was significantly greater than the effect of T1AM i.p. (Fig. 4b).

**Glucoregulatory hormones**  Similar to T1AM i.p. (Fig. 2a), neither T1AM- nor T0AM-administered i.c.v. induced a significant change in plasma insulin content (Fig. 5a). Although there was a trend for insulin to decrease 10 min after i.c.v. infusion of T1AM, this response failed to achieve statistical significance ($P < 0.063$; Fig. 5a).

Plasma glucagon increased by 155% (from 69 ± 10 to 176 ± 20 pg/ml) 10 min after i.c.v. T1AM infusion ($P < 0.0001$ versus Veh). During the same time period, T0AM i.c.v. also significantly increased plasma glucagon, but only by 58% ($P = 0.004$ versus Veh). Interestingly, the magnitude of T1AM's impact on circulating glucagon levels was dependent on the route of administration with i.c.v. infusion producing a greater effect in the first 10 min than i.p. administration (108 ± 21 vs 50 ± 11 pg/ml respectively; $P = 0.044$). Unlike the sustained elevation that followed i.p. administration of either T1AM or T0AM, plasma glucagon returned to basal levels within 60 min of infusing either compound i.c.v. (Fig. 5b).

Plasma corticosterone concentrations were significantly increased following i.c.v. infusion of either 0.5 mg/kg T1AM or T0AM (Fig. 5c) with both treatments producing nearly equivalent maximum effects by $t = 20$ min post-injection (delta corticosterone $t = 20$ vs $t = 0$: 6 ± 44 ng/ml Veh i.c.v., 206 ± 58 ng/ml T1AM i.c.v., 296 ± 49 ng/ml T0AM i.c.v., $P = 0.02$ T1AM versus Veh, $P < 0.0001$ T0AM versus Veh).

Plasma T3, T4, and TSH concentrations before and after central (i.c.v.) infusion of either T1AM, T0AM, or vehicle (saline-DMSO) infusion are shown in Table 2. Although plasma T3, T4, and TSH were found to decrease in all treatment groups by 120 min post-i.c.v. bolus infusion, neither i.c.v. T1AM nor T0AM had a statistically significant effect on plasma T3, T4, or TSH compared with vehicle i.c.v.

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**Figure 2** (a) Plasma insulin concentration before ($t = 0$) and after i.p. injection of T1AM, T0AM, or vehicle. ANOVA indicates no effects of time and no differences in insulin concentration between groups (ANOVA RM factor $time = 0.556$, $time \times group = 0.735$, and $group = 0.213$). (b) Plasma glucagon concentration before ($t = 0$) and after i.p. injection of T1AM, T0AM, or vehicle. From $t = 10$, glucagon is higher in both T1AM- and T0AM-treated rats relative to vehicle rats ($^* P < 0.05$ versus Veh). At $t = 60$, glucagon concentration is higher in T1AM when compared with T0AM-treated rats ($^* P = 0.05$ T0AM versus T1AM). ANOVA RM factor $time = 0.0001$, $time \times group = 0.0001$, and $group = 0.001$. (c) Plasma corticosterone concentration before ($t = 0$) and after ($t = 5–120$ min) i.p. injection of T1AM, T0AM, or vehicle. T0AM-treated animals have higher plasma corticosterone from $t = 30$ and T1AM-treated animals only on $t = 60$ when compared with vehicle ($^* P < 0.05$ versus Veh). Note that at no time-point, plasma corticosterone between T1AM- and T0AM-injected animals differs. ANOVA RM factor $time = 0.0001$, $time \times group = 0.050$, and $group = 0.030$. 

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Table 1  Plasma thyroid hormone concentrations before (basal) and after (2 h) i.p. vehicle, T₁AM, and T₀AM infusion

<table>
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<th>Veh i.p. n=7</th>
<th>T₁AM i.p. n=7</th>
<th>T₀AM i.p. n=8</th>
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<td>Basal 2 h</td>
<td>Basal 2 h</td>
<td>Time Time× Group Group</td>
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<td>T₃ (nmol/l)</td>
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<td>0.78±0.05</td>
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<td>T₄ (nmol/l)</td>
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<td>70±4*</td>
<td>91±4</td>
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<td>TSH (mU/l)</td>
<td>1.46±0.27</td>
<td>2.03±0.31</td>
<td>1.79±0.25</td>
<td>0.028</td>
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*P<0.05 versus basal value within the same group, †P<0.05 versus Veh basal, ‡P<0.05 versus Veh 2 h, §P<0.05 versus T₁AM 2 h.

Discussion

In an effort to determine whether the thyronamines T₁AM and T₀AM can affect glucose homeostasis by acting directly on the brain, we compared their physiologic consequences following systemic and central administration. The major finding of our study is that central administration of low-dose (i.e. 1% of the systemic dose) T₁AM acutely increases EGP and plasma glucose concentration to a similar – or even greater – extent compared with systemic T₁AM, concomitant with an increase in plasma glucagon and corticosterone concentrations. Similar effects were observed following central T₀AM infusion, albeit to a lesser extent. When administered intravenously, the same low dose of T₁AM and T₀AM, which was effective centrally, had no detectable effect on plasma glucose or EGP; thus excluding the possibility that the observed responses were the result of leakage of the centrally administered compound into the circulation and acting peripherally.

Rats infused intraperitoneally with T₁AM, and to a lesser extent T₀AM, exhibited a behavioural phenotype within minutes of administration, which was remarkably similar to the fully reversible behavioural changes reported earlier in mice (Scanlan et al. 2004, Doyle et al. 2007). In short, animals exhibited a decrease in overall locomotor activity and responsiveness to external stimuli (visual and auditory), while reflexes were preserved. Furthermore, the hyperglycemia that develops in mice (Regard et al. 2007) following thyronamine exposure also is seen in rats (Fig. 1a). Moreover, we show for the first time that the hyperglycemia induced by the thyronamines T₁AM and (to a lesser extent) T₀AM occurs simultaneously with a rapid (i.e. within 10 min), ~50% increase in EGP that was maintained for the duration of the experiment (Fig. 1b).

With respect to the systemic administration of thyronamines, there are several mechanisms that may contribute to the alterations in glucose metabolism we observed. First, plasma glucagon increases rapidly in response to systemic T₁AM and T₀AM administration, concomitant with the increase in plasma glucose and EGP. It was expected that the thyronamine-induced hyperglycemia (up to 22 mmol/l; Fig. 1a) would provoke a considerable insulin response. However, plasma insulin did not change in spite of the overt hyperglycemia produced by either thyronamine. The plasma glucagon increase together with this inadequate insulin response is likely to be causal factors in the increase in plasma glucose and EGP induced by thyronamines. These effects on
Central thyronamines and glucose metabolism

With regard to the possible mechanisms underlying the effects of centrally administered thyronamines on glucose metabolism, it is interesting that concomitant with the rapid increase in EGP (Fig. 3b), plasma insulin levels tended to decrease acutely after central administration of 0.5 mg/kg T1AM (Fig. 5a) in contrast to systemically (i.e., i.p.) administered drug. In addition, after i.c.v. infusion of 0.5 mg/kg T1AM, there was a rapid increase in plasma glucagon (Fig. 5b), which was more pronounced than the early glucagon increase after systemic thyronamine infusion. No change in EGP, plasma insulin, and glucagon levels was observed after i.v. infusion of 0.5 mg/kg T1AM, confirming that T1AM-imposed actions on the CNS are causal to these phenomena. This dependence of thyronamine effects on the route of administration point to neural or (neuro)transmitter type, rather than humoral type of actions. Indeed, it has been demonstrated that T1AM modulates synaptosomal transport of neurotransmitters such as dopamine and norepinephrine (Snell et al. 2007), supposedly by behaving as endogenous monoamine reuptake inhibitors (Weatherman 2007). In addition, low-dose T1AM administration in the lateral cerebral ventricles and in the arcuate nucleus was recently reported to rapidly increase food intake (Dhillon et al. 2008). The effects of thyronamines on plasma insulin and glucagon in the present study may be explained by increased sympathetic tone in the pancreas, mediated via central thyronamine actions. In addition, centrally administered thyronamines might stimulate autonomic outflow from the hypothalamus to the liver thereby elevating EGP. In support of this conjecture is accumulating evidence demonstrating the brain’s important role, particularly the hypothalamus, in regulating hepatic glucose metabolism via sympathetic and parasympathetic projections to the liver (Obici et al. 2002, Kalsbeek et al. 2004, Pocai et al. 2005, Klieverik et al. 2008).

As thyronamines have been hypothesized to constitute a novel aspect of TH biology (Liggett 2004, Scanlan et al. 2004), it was of interest to assess how thyroid-related parameters in euthyroid animals responded to synthetic thyronamines. Systemic infusion of these compounds, in particular T1AM, depressed plasma TSH, T4, and T3 levels, whereas central administration had no such effect. These responses could represent a state reminiscent of the non-thyroidal illness syndrome (Fliers et al. 1997, Boelen et al. 2004, Adler & Wartofsky 2007). Although it is conceivable that the thyronamines altered TH secretion by decreasing TSH release from the pituitary, the observation that central thyronamine administration does not induce plasma TH alterations relative to vehicle, argues against this possibility. Finally, an effect of thyronamines on plasma concentrations of iodothyronines via interaction with the deiodinase enzymes seems less likely as T1AM does not interfere with D1-mediated iodothyronine deiodination in vitro (Piehl et al. 2008).

Systemic thyronamine administration produced significant increases in plasma corticosterone levels that were similar in T1AM- and T0AM-infused rats (Fig. 2c), and could be recapitulated by low-dose (0.5 mg/kg) i.c.v. infusion of

plasma glucagon and insulin might be explained by direct actions of T1AM and T0AM on the pancreatic α and β-cells, supposedly by binding to GPCRs such as TAAR1 or ARα2 (Liggett 2004, Scanlan et al. 2004). Indeed, pharmacological stimulation of ARα2 has been shown to induce hyperglycemia and inhibit insulin release (Angel et al. 1990). Second, given the rapid onset of thyronamine-induced changes, it is possible that T1AM and T0AM activation of GPCRs expressed in hepatocytes underlies the stimulation of EGP we observed, analogous to the stimulation of β-ARs by norepinephrine.

Figure 4 (a) Changes in plasma glucose concentration (delta) before and after i.p. versus i.c.v. bolus infusion of T1AM (T1AM i.p. and T1AM i.c.v.) or vehicle (Veh i.p. and Veh i.c.v.). From t = 5, glucose concentration in T1AM i.p. and T1AM i.c.v. groups is increased compared with respective vehicle groups (*P < 0.05). Note that on t = 20, 30, and 45 min, glucose concentration in T1AM i.p. and T1AM i.c.v. rats is not different. The changes in plasma glucose concentration are expressed as the difference compared to the plasma glucose concentration at t = 0 min for each individual animal. ANOVA RM factor time P < 0.0001, time X group P < 0.0001, and group P < 0.0001. *P < 0.05; T1AM i.p. versus T1AM i.c.v. (b) Changes in endogenous glucose production (EGP) before and after i.p. versus i.c.v. bolus infusion of T1AM (T1AM i.p. and T1AM i.c.v.) or vehicle (Veh i.p. and Veh i.c.v.). From t = 5 and t = 10 min, EGP in T1AM i.c.v. and T1AM i.p.-infused rats respectively is increased when compared with respective vehicle groups (P < 0.05). Note that on t = 30, EGP is higher in T1AM i.c.v. than in T1AM i.p.-treated rats (*P < 0.05). The changes in EGP are expressed as the difference compared to EGP at t = 0 min for each individual animal. ANOVA RM factor time P < 0.0001, time X group P < 0.0001, and group P < 0.0001.
synthetic T1AM or T0AM (Fig. 5c), suggesting that these represent central effects of thyronamines on the hypothalamus–pituitary–adrenal axis. The elevated corticosterone could contribute in a limited way to the hyperglycemic state but, more importantly, because in both experiments the hyperglycemia was much more pronounced in T1AM when compared with T0AM-infused animals, it is unlikely to account for the major glucose increase induced by systemic and central T1AM.

There are several mechanisms by which circulating thyronamines might exert their actions in the CNS. First, there could be passive or active transport of circulating thyronamines across the blood–brain barrier, the latter analogous to iodothyronines (Dratman et al. 1991). Second, circulating thyronamines might bind cell-surface receptors in the plasma membrane of neurons located in circumventricular nuclei such as the arcuate nucleus where the blood–brain barrier is absent. The arcuate nucleus is known to mediate central actions of the peptide hormones like insulin and leptin via locally expressed leptin and insulin receptors (Niswender & Schwartz 2003), and TAAR1 is expressed in the arcuate nucleus (Borowsky et al. 2001). However, our finding that the EGP increase is not as robust following central administration of thyronamines as it is after systemic administration suggests their central actions alone are insufficient to account for the persistent EGP increase and hyperglycemia. Consistent with this interpretation are the results from a recent study in which mice pre-treatment with 6-hydroxydopamine still developed hyperglycemia and hypoinsulinemia following i.p. administration of T1AM, suggesting that these T1AM-induced alterations can occur in the absence of sympathetic signalling (Regard et al. 2007). Another possibility is that thyronamines impose both peripheral and central actions on glucose metabolism, occurring independently. In this intriguing scenario, central actions could be mediated by thyronamines formed locally in the brain, by conversion from iodothyronines such as T4, T3, and/or reverse T3.

The rapid and pronounced metabolic effects produced by central administration of T1AM and T0AM suggest that one or more receptors mediate their actions. Indeed, T1AM and T0AM dose dependently activate the Gs protein-coupled TAAR1 receptor (Borowsky et al. 2001, Bunzow et al. 2001, Grandy 2007, Wainscott et al. 2007). TAAR1 belongs to a large family of related receptors (Borowsky et al. 2001, Grandy 2007), and this receptor’s mRNA is expressed in a wide variety of tissues including many areas of the brain.

Figure 5 (a) Plasma insulin concentrations before (t=0) and after i.c.v. bolus infusion of T1AM, T0AM, or vehicle. Note that at t=10 min, there is a trend for insulin concentrations to be depressed in animals receiving 0.5 mg/kg T1AM i.c.v. compared with vehicle-treated rats (*P=0.063). ANOVA RM factor time P<0.01, time×group P>0.161, and group P>0.758. (b) Plasma glucagon concentrations before (t=0) and after i.c.v. bolus infusion of T1AM, T0AM, or vehicle. At t=10 min, glucagon is significantly higher in T1AM i.c.v. and, to a lesser extent, T0AM i.c.v. when compared with vehicle i.c.v.-treated rats. *P<0.01 versus vehicle i.c.v., ^P<0.01 T1AM versus T0AM i.c.v. ANOVA RM factor time P<0.0001, time×group P<0.0001, and group P<0.0001. (c) Plasma corticosterone concentration before (t=20 and 0 min) and after (t=5 to t=120 min) i.c.v. bolus infusion of T1AM, T0AM, or vehicle. Circulating corticosterone levels rapidly increase following the infusion of T1AM or T0AM at t=20 and t=10 min when compared with vehicle respectively. Note that T1AM- and T0AM i.c.v.-infused groups do not differ at any time point, except for t=5 min. *P<0.05 versus vehicle i.c.v., ^P<0.01 T1AM versus T0AM i.c.v. ANOVA RM factor time P<0.0001, time×group P=0.002, and group P=0.001.
precursor iodothyronines (i.e. T3, T4, and rT3), the enzymes in the literature for synthesized. Although there is currently no direct evidence in important to establish how and where these compounds are namines remains to be addressed. In this context, it will be Therefore, the biological significance of endogenous thyro-

Chiellini et al. administration of synthetic material (Braulke 2005), supporting their possible involvement in mediating hypothalamic regulation of sympathetic outflow (Li et al. 2005), indicating that thyronamines can act centrally in order to modulate glucose metabolism.

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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References


Borowsky et al. 2001, Bunzow et al. 2001). The fact that the rank order of potency as a TAAR1 agonist in vitro, T1AM being more potent than T0AM (Scanlan et al. 2004), is also reflected in the metabolic responses described in the present study, fits with the notion that TAAR1 may mediate some actions of T1AM and T0AM. In addition, Regard et al. (2007) have recently shown that whereas T1AM induces hyperglycemia after systemic administration in wild-type mice, this effect is lost in ARα2-deficient mice as well as mice pre-treated with the ARα2 antagonist yohimbine. Moreover, by using a transgenic approach, they provided strong evidence that the hyperglycemia and concurrent hypoinsulinemia following T1AM infusion in mice was dependent upon pancreatic Gi protein-coupled receptor expression. Collectively, these data suggest that, at least for the effects of systemically administered T1AM on glucose metabolism, ARα2 is important. Interestingly, ARα2 are highly expressed in the hypothalamus as well and contribute to the hypothalamic regulation of sympathetic outflow (Li et al. 2005), supporting their possible involvement in mediating effects of centrally administered thyronamines.

To date, every published metabolic and physiologic study involving thyronamines, including this one, has relied on the administration of synthetic material (Braulke et al. 2007). Therefore, the biological significance of endogenous thyronamines remains to be addressed. In this context, it will be important to establish how and where these compounds are synthesized. Although there is currently no direct evidence in the literature for in vitro conversion of thyronamines from precursor iodothyronines (i.e. T3, T4, and rT3), the enzymes indispensable for such conversion such as aromatic amino acid decarboxylase for decarboxylation and iodothyronine dioxygenases type 2 and 3 for deiodination are widely distributed in the CNS, and indeed within the hypothalamus (Zhu & Juorio 1995, Tu et al. 1997, Alkemade et al. 2005, Lecham & Fekete 2005). An important remaining question is whether the metabolic effects in the present and other studies represent physiological or pharmacological effects of thyronamines. The systemic dose of T1AM used in our study has been shown to induce a tenfold increase in plasma T1AM concentration within 3 h after infusion in Siberian hamsters (Braulke et al. 2007). However, there are currently no data on the pharmacokinetic characteristics (distribution volume, clearance, and binding to plasma proteins) of thyronamines, and at present it is unknown how thyronamine tissue concentrations during experimental manipulations compare to their concentrations under more physiologic conditions.

We conclude that central administration of a low dose of either T1AM or T0AM can acutely induce increased EGP and hyperglycemia, concomitant with increased plasma glucagon, corticosterone, and a deficient insulin response. These changes are very similar to the acute changes observed after systemic T1AM and T0AM administration. Our data indicate that thyronamines can act centrally in order to modulate glucose metabolism.

Table 2

<table>
<thead>
<tr>
<th>Veh i.c.v. n=8</th>
<th>T1AM i.c.v. n=9</th>
<th>T0AM i.c.v. n=8</th>
<th>ANOVA RM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td>Time</td>
<td>Time</td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>2 h</td>
<td>Basal</td>
<td>2 h</td>
</tr>
<tr>
<td>T3 (nmol/l)</td>
<td>1.27±0.08</td>
<td>0.77±0.05*</td>
<td>1.39±0.15</td>
</tr>
<tr>
<td>T4 (nmol/l)</td>
<td>81±7</td>
<td>53±4*</td>
<td>79±7</td>
</tr>
<tr>
<td>TSH (mU/l)</td>
<td>1.64±0.33</td>
<td>0.77±0.16*</td>
<td>1.36±0.26</td>
</tr>
</tbody>
</table>

*P≤0.05 versus basal value within the same group.

Time

Basal 2 h Basal 2 h Basal 2 h

T3 (nmol/l) 1.27±0.08 0.77±0.05* 1.39±0.15 0.86±0.09*

Time

ANOVA RM

P p p

Time× Group

pp

*P≤0.05 versus basal value within the same group. (Braulke et al. 2007, Doyle et al. 2007, Regard et al. 2007). The fact that the rank order of potency as a TAAR1 agonist in vitro, T1AM being more potent than T0AM (Scanlan et al. 2004), is also reflected in the metabolic responses described in the present study, fits with the notion that TAAR1 may mediate some actions of T1AM and T0AM. In addition, Regard et al. (2007) have recently shown that whereas T1AM induces hyperglycemia after systemic administration in wild-type mice, this effect is lost in ARα2-deficient mice as well as mice pre-treated with the ARα2 antagonist yohimbine. Moreover, by using a transgenic approach, they provided strong evidence that the hyperglycemia and concurrent hypoinsulinemia following T1AM infusion in mice was dependent upon pancreatic Gi protein-coupled receptor expression. Collectively, these data suggest that, at least for the effects of systemically administered T1AM on glucose metabolism, ARα2 is important. Interestingly, ARα2 are highly expressed in the hypothalamus as well and contribute to the hypothalamic regulation of sympathetic outflow (Li et al. 2005), supporting their possible involvement in mediating effects of centrally administered thyronamines.

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