A model for chronic, intrahypothalamic thyroid hormone administration in rats

Z Zhang¹, P H Bisschop¹, E Foppen¹, H C van Beeren¹, A Kalsbeek^{1,2}, A Boelen¹ and E Fliers¹

¹Department of Endocrinology and Metabolism, Academic Medical Center (AMC), University of Amsterdam, Amsterdam, the Netherlands

²Hypothalamic Integration Mechanisms, Netherlands Institute for Neuroscience (NIN), Amsterdam, Amsterdam, the Netherlands

Correspondence should be addressed to Z Zhang

Email
z.zhang@amc.uva.nl

Abstract

In addition to the direct effects of thyroid hormone (TH) on peripheral organs, recent work showed metabolic effects of TH on the liver and brown adipose tissue via neural pathways originating in the hypothalamic paraventricular and ventromedial nucleus (PVN and VMH). So far, these experiments focused on short-term administration of TH. The aim of this study is to develop a technique for chronic and nucleus-specific intrahypothalamic administration of the biologically active TH tri-iodothyronine (T₃). We used beeswax pellets loaded with an amount of T₃ based on *in vitro* experiments showing stable T₃ release (~5 nmol l⁻¹) for 32 days. Upon stereotactic bilateral implantation, T₃ concentrations were increased 90-fold in the PVN region and 50-fold in the VMH region after placing T₃-containing pellets in the rat PVN or VMH for 28 days respectively. Increased local T₃ concentrations were reflected by selectively increased mRNA expression of the T₃-responsive genes *Dio3* and *Hr* in the PVN or in the VMH. After placement of T₃-containing pellets in the PVN, *Tshb* mRNA was significantly decreased in the pituitary, without altered *Trh* mRNA in the PVN region. Plasma T₃ and T₄ concentrations decreased without altered plasma TSH. We observed no changes in pituitary *Tshb* mRNA, plasma TSH, or plasma TH in rats after placement of T₃-containing pellets in the VMH. We developed a method to selectively and chronically deliver T₃ to specific hypothalamic nuclei. This will enable future studies on the chronic effects of intrahypothalamic T₃ on energy metabolism via the PVN or VMH.

Key Words

- ▶ PVN
- ▶ VMH
- ▶ hypothalamus
- ▶ HPT axis
- ▶ T₃

Journal of Endocrinology
(2016) **229**, 37–45

Introduction

Thyroid hormone (TH) is a major determinant of basal metabolic rate as well as glucose and lipid metabolism (Mullur *et al.* 2014, Sinha *et al.* 2014). A number of recent studies have shown that in addition to the well-known direct effects of TH at the tissue level, some of its metabolic effects are mediated indirectly through specific neuronal targets within the hypothalamus. In particular, this has been shown in rats for hepatic glucose metabolism, brown adipose tissue (BAT) function, and the central

control of blood pressure and heart rate (for reviews see Fliers *et al.* 2010, Warner & Mittag 2012, Lopez *et al.* 2013). Studies on rats have shown that thyrotoxicosis increases glucose production in the liver, while reducing hepatic insulin sensitivity. These effects appeared to be modulated by selective hepatic sympathetic and parasympathetic denervation (Klieverik *et al.* 2008), and follow-up experiments showed that TH stimulates hepatic glucose production via a sympathetic pathway

originating in the hypothalamic paraventricular nucleus (PVN) (Klieverik *et al.* 2009). Similarly, indirect effects of TH on BAT via the hypothalamic ventromedial nucleus (VMH) were reported by several groups. First, mice heterozygous for a mutant TR α 1 with low affinity for T₃ appeared to be hypermetabolic due to increased BAT activity, resulting in increased thermogenesis and energy expenditure. The metabolic phenotype was blunted after a functional denervation of sympathetic signaling, suggesting that the central nervous system (CNS) controlled the hypermetabolism of these mice through the autonomic nervous system (Sjogren *et al.* 2007). More recently, López and coworkers showed that activation of the thermogenic program in BAT through the sympathetic nervous system (SNS) depends on T₃-mediated activation of *de novo* lipogenesis in the hypothalamic VMH (Lopez *et al.* 2010), establishing a role for T₃ in the VMH in the regulation of BAT. Together, these studies revealed a distinct role of hypothalamic T₃ in the regulation of peripheral organs through the autonomic nervous system.

It should be noted that the above-mentioned metabolic effects of intrahypothalamic T₃ were observed in acute experiments, i.e., a few hours after T₃ administration, whereas metabolic dysfunction in thyrotoxicosis, including insulin resistance and weight loss, is mostly due to long-term exposure to excessive TH. Thus, the relationship between chronically elevated intrahypothalamic T₃ concentrations and metabolic dysfunction, which may be of clinical relevance, has not been reported yet. Before embarking on experiments to explore this relationship, we aimed to develop a method for selective and chronic (4 weeks) administration of T₃ in the rat PVN or VMH region.

We implanted T₃-containing or control pellets bilaterally into either the PVN or the VMH region of rats for a period of 28 days. Plasma T₃, T₄, and TSH concentrations were measured at regular time intervals after placement of the pellets. Hypothalamic tissue punches containing either the PVN or the VMH, as well as pituitary glands, were collected at the end of the experiment for the assessment of local T₃ tissue concentration and gene expression analysis. We conclude from our experiments that it is feasible to deliver T₃ selectively and chronically to distinct hypothalamic nuclei.

Materials and methods

Preparation of pellets

3,3',5-tri-iodo-L-thyronine (Sigma-Aldrich) was mixed with melted beeswax (Sigma-Aldrich) at a ratio of 1:9 (w/w) in a

70°C water bath. The mixture was packaged into a length of 1mm cylindrical pellet using a 23G stainless cannula. The weight of the pellets was around 76.8µg, including 10% of T₃ amounting to 7.68µg. Blank beeswax pellets without T₃ were used as control pellet. During surgery, the pellet was extruded out of the cannula by a stainless steel wire plunger.

In vitro experiment

T₃-containing pellets (T₃Ps) or control pellets (cPs) were incubated in 500µl bovine serum on a 37°C homeothermic shaker for 28 days. After 1 day and every 3 days thereafter, a sample of 250µl serum was taken from the system for assessment of T₃ concentration and replaced by an equal amount of fresh serum.

Animal experiments

Male Wistar rats weighting 280–320 g (total number 92; Charles River Breeding Laboratories, Sulzfeld, Germany) were housed individually in a 12h light:12h darkness cycle environment. Chow and water were provided *ad libitum*. All procedures were approved by the Animal Care Committee of the Royal Netherlands Academy of Arts and Sciences.

After 1 week of acclimatization in the facility, a total number of 84 animals divided into eight groups (see Table 1) were anesthetized by an intramuscular injection of Hypnorm (fentanyl, 0.252mg/kg BW; fluanisone, 8mg/kg BW; Janssen, High Wycombe, Buckinghamshire, UK) followed by a subcutaneous Midazolam (1mg/kg BW; Roche) injection. T₃Ps or cPs were implanted bilaterally into the hypothalamic PVN (anteroposterior: –1.8 mm; lateral: 2.1 mm; ventral: –6.9 mm; angle: 10°) or VMH (anteroposterior: –2.3 mm; lateral: 2.2 mm; ventral: –8.1 mm; angle: 10°) region using a standard Kopf stereotaxic apparatus (Paxinos & Watson 2005). Pellet placements were checked in 20µm cresyl violet-stained brain sections of each animal at the end of the experiment. Blood samples (around 250µl) were taken by tail incisions using heparin-coated capillary Microvette (Sarstedt AG & Co, Nümbrecht, Germany) 3 days before and 3, 7, 14, 21, and 28 days after pellet placement. The plasma samples were collected after centrifugation at 4000rpm for 15 min and stored at –20°C. After 28 days, rats were decapitated; trunk blood was taken and stored at –20°C until analysis. Pituitaries were snap frozen in liquid nitrogen and stored in –80°C until further use.

Table 1 Design of the experimental groups.

No.	Group	Treatment	Group size	Description
1	Intact	Untreated	8	For qPCR in hypothalamic punches
2	cP_PVN	Control pellets in the PVN	8	
3	T ₃ P_PVN	T ₃ -containing pellets in the PVN	12	
4	cP_VMH	Control pellets in the VMH	8	
5	T ₃ P_VMH	T ₃ -containing pellets in the VMH	12	For T ₃ brain tissue concentrations
6	cP_PVN	Control pellets in the PVN	11	
7	T ₃ P_PVN	T ₃ -containing pellets in the PVN	11	
8	cP_VMH	Control pellets in the VMH	11	
9	T ₃ P_VMH	T ₃ -containing pellets in the VMH	11	

Brains were removed and frozen on dry ice. The hypothalamus was cut into 300 µm coronal sections using a cryostat (Leica CM1950). For T₃ tissue concentration and gene expression measurements, the PVN and VMH regions were collected by punching the area with a 17G stainless steel needle according to Palkovits (1973). The third ventricle and major fiber tracts served as landmarks for orientation. Tissue was then homogenized either in lysis buffer (Roche Applied Science, Penzberg, Germany) for RNA isolation or in PBS for the assessment of tissue T₃ concentration. Validation of the punched blocks was performed by measuring the expression levels of nucleus-specific genes by qPCR.

Plasma thyroid hormones

Plasma T₃ and T₄ concentrations were determined using an in-house RIA as reported before (inter-assay variation T₃, 6.2% and T₄, 7.3%; intra-assay variability T₃, 3.6% and T₄, 6.6%) (Wiersinga & Chopra 1982). Plasma TSH was determined by a chemiluminescent immunoassay, using the Immulite 2000 (Siemens). For TSH, the inter-assay variation was ±9% and the intra-assay variation was 3–4%.

Within the same experiment, all samples were measured within one run to prevent inter-assay variation.

Hypothalamic tissue T₃ concentrations

The PVN and VMH punches were weighed and homogenized in 300 µl methanol using a Magna Lyser (Roche Molecular Biochemicals, Mannheim, Germany). The homogenates were then extracted using a double volume of chloroform. The mixtures were centrifuged at 3184g at 4°C for 15 min. Supernatants from the extractions were transferred into glass vials and vaporized under nitrogen gas for 30 min. Subsequently, samples were dissolved in 70 µl alkaline T₃ RIA assay buffer and 70 µl T₃-free plasma. T₃ concentrations were measured by T₃ RIA as described above.

RNA isolation and real-time PCR (qPCR)

Total RNA from pituitary, the PVN, and VMH punches were isolated using the MagNA Pure LC 2.0 Instrument (Roche Molecular Biochemicals) with Magna pure tissue III total RNA kit (Roche Molecular Biochemicals). RNA yield was determined using the Nanodrop (Nanodrop),

Table 2 Primers used for qPCR.

Gene	symbol	Forward (5'-3')	Reverse (5'-3')	Products length (bp)
Glyceraldehyde-3-phosphate dehydrogenase	<i>Gapdh</i>	TGAACGGGAAGCTCACTGG	TCCACCACCCTGTTGCTGTA	306
Hypoxanthine guanine phosphoribosyl transferase	<i>Hprt</i>	GCAGTACAGCCCCAAAATGG	AACAAAGTCTGGCCTGTATCCAA	84
Thyrotropin releasing hormone, prepropeptide	<i>Trh</i>	TCTGCAGAGTCTCCACTTCG	AGAGCCAGCAGCAACCAA	59
Deiodinase type 3	<i>Dio3</i>	AGCGCAGCAAGAGTACTTTCAG	CCATCGTGTCAGAACCAAG	61
Hairless	<i>Hr</i>	AGTTTGACATTTCGGGGGCAC	TGGGGGAGTTTTCTCTGTTGG	101
Steroidogenic factor 1 (NR5A1)	<i>Sf1</i>	CCAGTGTCCACCCTTATCCG	ACCTTGTCACCACACACTGG	117
Oxytocin	<i>Oxt</i>	TGCGCAAGTGCTTCCCTGCG	AGCCATCCGGGCTACAGCAGA	216
Thyroid-stimulating hormone, β subunit	<i>Tshb</i>	TCGTTCTCTTTCCGTGCTT	CGGTATTTCCACCGTTCTGT	245

Figure 1

T₃-containing pellets result in rather stable T₃ concentrations in the medium for >30 days. Open circles denote control pellets (cP) and solid circles denote T₃-containing pellets (T₃P). Mean $\pm$ S.E.M. of three pellets/group are depicted.

and cDNA was synthesized with equal RNA input with the First-Strand cDNA synthesis kit (AMV) for qPCR with oligo-d (T) primers (Roche Molecular Biochemicals). As a control for genomic DNA contamination, a cDNA synthesis reaction without reverse transcriptase was included. Quantitative PCR was performed using the LightCycler 480 (Roche Molecular Biochemicals) and LightCycler 480 SYBR Green I Master mix (Roche Molecular Biochemicals). The primers used for qPCR are listed in Table 2. Quantification was performed using the LinReg software. PCR efficiency was checked individually and samples with a deviation of more than 5% of the mean were excluded

from the analysis. Calculated values were related to the geometric mean expression of *Gapdh* and *Hprt*, reference genes showing stable expression under the experimental conditions.

Statistical analysis

Data are expressed as mean $\pm$ standard error of the mean (S.E.M.). Differences between two groups were analyzed using an independent two-tailed Student's t-test. When data were not normally distributed, the independent non-parametric Mann-Whitney U test was used. The changes in T₃ and T₄ concentrations in the plasma were evaluated by two-way ANOVA with repeated measurements followed by Bonferroni *post hoc* analysis. Statistical significance was defined at a level of $p < 0.05$.

Results

In vitro experiments

Before the *in vivo* experiments, we tested the release of T₃ from T₃P in serum. The *in vitro* results showed that T₃ was constantly released into the medium at a concentration of ~ 5 nmol l⁻¹ from the T₃P (corresponding to a T₃ release of around 0.26 ng per day), but not from cP for a period of 32 days (Fig. 1).

Figure 2

Representative photomicrographs of brain sections, showing the location and size of the pellet implants (A) and of the micro-punches (B) in the PVN (left panel) and VMH (right panel). Scale bars represent 1 mm. (C) shows *Oxt* mRNA expression in the PVN and VMH punches and (D) shows *Sf1* mRNA expression in the PVN and VMH punches. $n = 8$ per group.

Figure 3
Local T₃ concentrations (nmol g⁻¹ brain tissue) in the PVN and VMH regions. (A) pellets were placed in the PVN, (B) pellets were placed in the VMH. *n* = 8–9 per group. **P* < 0.05, ****P* < 0.001.

Pellet placement and validation of hypothalamic dissection

T₃Ps or cPs were placed either in the PVN or in the VMH. Post-mortem examination revealed that the majority of pellets were placed laterally from the PVN and dorsolaterally in the VMH with an anteroposterior location ranging from -1.0 to -1.7 mm from bregma (Fig. 2). Nine animals were excluded from the analysis because of the misplacement of the pellets. In the animals implanted with cPs, no adverse effects were found in terms of food and water intake or growth compared with an intact, non-operated control group (*n* = 8). In addition, no differences were found in gene expressions (i.e., *Dio3*, *Hr*, and *Trh* in hypothalamus and *Tshb* in pituitary) or plasma TSH, T₃, and T₄ concentrations between these two groups (data not shown).

In order to validate the anatomy of the punches, we assessed oxytocin (*Oxt*) mRNA, which is PVN-specific

(Swanson & Sawchenko 1983) and steroid factor 1 (*Sf1*) mRNA, which is VMH-specific (Roselli *et al.* 1997). As shown in Fig. 2C, *Oxt* mRNA was expressed 1000-fold less in the VMH compared with the PVN. In contrast, *Sf1* mRNA expression was expressed over 20-fold higher in the VMH compared with the PVN (Fig. 2D), supporting the selectivity of the anatomical punches.

Tissue T₃ concentrations in the PVN and VMH

We then evaluated the local T₃ concentration after 28 days of implantation in micro-dissected PVN and VMH punches. As expected, local T₃ concentrations were much higher in the T₃P-treated group compared with the cP group. T₃ concentrations increased 90-fold in the PVN region and 50-fold in the VMH region compared with the cP group when the T₃Ps were placed in the PVN or VMH respectively (Fig. 3). When T₃Ps were placed in the PVN, we also observed increased T₃ in the VMH compared with the cP group (Fig. 3A), although to a much lesser extent than in the PVN. When T₃Ps were placed in the VMH, no increased T₃ concentrations in the PVN were observed. In fact, T₃ concentrations were even significantly lower compared with the cP group (Fig. 3B).

Differential effect of T₃ administration in the PVN and VMH on T₃-responsive genes

We assessed the effects of local T₃ administration according to the mRNA expression of T₃-responsive genes in the targeted nuclei. When implanting the T₃Ps in the PVN, both *Dio3* and *Hr* mRNA expressions were significantly increased in the PVN region, whereas *Dio3* and *Hr* mRNA expressions in the VMH region were not affected (Fig. 4A and B). Conversely, placement of T₃Ps in the VMH induced a significant increase in *Dio3* and *Hr* mRNA in the VMH region, but not in the PVN region (Fig. 4C and D). Gene expression was not different between intact animals and animals implanted with cPs (data not shown).

Effect of T₃ administration in the PVN on HPT axis

Placement of T₃Ps in the PVN did not change *Trh* mRNA in the PVN or VMH region, while decreasing pituitary *Tshb* mRNA expression (*p* < 0.001) (Fig. 5A and B). There was no significant effect on plasma TSH concentrations (Fig. 5C). Plasma T₃ concentrations were significantly decreased in rats with T₃Ps in the PVN at day 3, 14, and 21 after implantation, whereas plasma T₄ concentrations were decreased at days 3 and 28 (Fig. 5D and E).

Figure 4

The mRNA expression of *Dio3* and *Hr* in the PVN and VMH. (A and B) Pellets placed in the PVN, (C and D) Pellets placed in the VMH. $n=8-10$ per group. * $P<0.05$, ** $P<0.01$.

Effect of T₃ administration in the VMH on HPT axis

Placement of T₃Ps in the VMH did not change *Trh* mRNA in the PVN; however, it decreased *Trh* mRNA in the VMH region (Fig. 6A). As expected, pituitary *Tshb* mRNA, plasma TSH (Fig. 6B and C), and plasma TH (Fig. 6D and E) concentrations did not change throughout the time course of this experiment.

Discussion

This study shows that it is feasible to selectively deliver T₃ for 4 weeks to the PVN or the VMH, without leakage of T₃ into the systemic circulation. Also within the hypothalamus, diffusion was limited, as T₃-containing pellets in the PVN did not affect T₃-responsive gene expression in the VMH or vice versa. As expected, chronic T₃ administration in the PVN, but not in the VMH, decreased pituitary *Tshb* mRNA expression as well as plasma T₃ and T₄ concentrations.

To set up the experimental model, we first performed pilot experiments *in vitro*. Incubation of T₃P in plasma resulted in rather constant T₃ concentrations in the physiological range for more than 1 month. Subsequently, we implanted the pellets into specific hypothalamic nuclei, followed by assessment of local tissue T₃ concentrations in hypothalamic tissue punches. The anatomical selectivity of the punch technique was confirmed by the selective expression of *Oxt* and *Sf1* in either the PVN or the VMH respectively. Twenty-eight days after placement of T₃Ps, T₃ concentrations increased 90-fold in the PVN region and

50-fold in the VMH region compared with cP when T₃Ps were placed in the PVN or VMH respectively. Although the local T₃ concentration was somewhat higher in the VMH after implantation of T₃Ps in the PVN, this did not induce T₃-responsive gene expression. These results show that T₃P released T₃ in a rather limited anatomical region for more than a month.

Next, we investigated the effects of T₃ administration by assessment of T₃-responsive gene expression. *Dio3* is a T₃-sensitive gene coding for a deiodinating enzyme that is critical in maintaining local TH homeostasis (Dentice & Salvatore 2011). As a main physiological inhibitor of TH action, it protects cells from excessive TH by converting T₃ (or T₄) into its inactive derivatives, T₂ (or T₃) (Dentice and Salvatore 2011). In rats, *Dio3* mRNA expression was shown to increase in the brain during hyperthyroidism, while it was undetectable during hypothyroidism (Tu *et al.* 1999, Bianco *et al.* 2002). In line with these observations, the present experiments showed that *Dio3* mRNA was only increased in the PVN but not in the VMH when the T₃Ps were placed in the PVN, while *Dio3* mRNA was only increased in the VMH but not in the PVN when the T₃Ps were placed in the VMH. Of note, *Dio3* mRNA expression may be enhanced by neuronal injury (Li *et al.* 2001). However, *Dio3* mRNA expression in non-operated rats did not differ from rats implanted with cPs, which excludes an effect of tissue damage by the pellet itself. In addition to *Dio3*, expression of *Hr*, which is another well-known T₃-responsive gene (Potter *et al.* 2002), was significantly increased only in the target nuclei where the T₃Ps were placed, again suggesting a nucleus-specific T₃

Figure 5

HPT axis response to T₃ administration in the PVN. (A) *Trh* mRNA in the PVN and VMH, (B) *Tshb* mRNA in pituitary gland, (C) plasma TSH, (D) Plasma T₃ and (E) Plasma T₄ concentrations. *n* = 8–10 per group. **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

response as a result of locally increased T₃ concentrations. A few additional reported T₃-responsive genes such as oxytocin (*Oxt*) (Dellovade *et al.* 1999) and monocarboxylate transporter 8 (*Mct8* (*Slc16a2*)) (Herwig *et al.* 2014) did not show significant changes between control and T₃-treated groups (data not shown). We did not observe any change in *Trh* mRNA expression measured by qPCR in a punch of the PVN region 28 days after placement of T₃Ps. A previous study using a hypothyroid rat model showed that unilateral T₃ crystalline implants within the anterior hypothalamus induced a marked reduction of *Trh* mRNA only in the medial parvocellular division of PVN as shown by *in situ* hybridization within 4 days (Dyess *et al.* 1988). Moreover, a study by Murphy *et al.* (2012) showed a mild suppression of hypothalamic *Trh* mRNA assessed by *in situ* hybridization 6 weeks after T₃ implants in the hypothalamus of Siberian hamsters. Therefore, the absence of an effect on *Trh* mRNA in the PVN after T₃ administration to the PVN in our study can probably be explained by the fact that only a subpopulation of TRH neurons in the PVN, i.e., the hypophysiotropic TRH neurons, are responsive to T₃ (Segerson *et al.* 1987, Fekete & Lechan 2014). In our approach, using the whole PVN region for qPCR, selective changes in the subpopulation of

hypophysiotropic TRH neurons may have gone unnoticed as all TRH neurons in the micro-punch are included in the PCR assessment.

Pituitary *Tshb* mRNA was lower after 28 days in the PVN in T₃P group compared with rats with cPs, in accordance with negative feedback regulation (Fliers *et al.* 2014). Plasma TSH concentration, however, was not significantly altered. Of note, a previous study showed that T₃ crystalline implants in the hypothalamus did not influence TSH secretion in hypothyroid rats, although IR-pro-TRH was remarkably inhibited (Dyess *et al.* 1988). The production and secretion of TSH by the pituitary gland are positively stimulated by TRH, while TRH also modulates the glycosylation of TSH, which affects TSH bioactivity (Magner 1990). Part of the explanation for unaltered plasma TSH in the presence of decreased pituitary *Tshb* mRNA as observed in this study may be a discrepancy between the biological activity and the immunological detectability of TSH (Persani & Bonomi 2014).

In line with negative feedback at the central level and decreased pituitary *Tshb* mRNA after T₃Ps implantation in the PVN, plasma T₃ and T₄ concentrations were significantly decreased throughout the experiment. In summary, our results indicate that the T₃Ps implantation in the PVN

Figure 6

HPT axis response to T₃ administration in the VMH. (A) *Trh* mRNA in PVN and VMH regions, (B) *Tshb* mRNA in pituitary gland, (C) Plasma TSH, (D) Plasma T₃ and (E) Plasma T₄ concentrations. *n* = 8–10 per group. **P* < 0.05.

delivers T₃ locally for at least 28 days. As an autonomic nervous system integration center, the PVN is involved in many aspects of energy metabolism, including hepatic glucose production. The investigation of metabolic effects of chronic intrahypothalamic T₃ administration in the PVN will be the focus of our future experiments.

Placement of T₃Ps in the VMH did not affect the HPT axis in any way, again supporting a locally restricted T₃ administration. Recent experiments have shown that TH in the VMH is a powerful determinant of BAT activity (Lopez *et al.* 2013). Thus, we expect that the present technique will enable studies on long-term metabolic consequence of central T₃ on energy balance in addition to glucose metabolism. Finally, as a recent study showed a novel central role for T₃ in cardiovascular regulation by acting on a group of pre-autonomic neurons in the anterior hypothalamus (AH) (Mittag *et al.* 2013), our model may be helpful to study the long-term effects of T₃ in the AH on blood pressure and heart rate as well.

In conclusion, our pilot experiments provide a promising technique to selectively deliver T₃ for at least 4 weeks to specific hypothalamic nuclei. The use of these T₃-containing pellets holds promise for studies on

metabolic effects of nucleus-specific chronic T₃ delivery within the hypothalamus.

Declaration of interest

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

Funding

This project was funded by a grant from the China Exchange Program (CEP) of the Royal Netherlands Academy of Sciences and the Chinese Academy of Sciences (grant #11CDP001, to E.F.) and by a grant from the Chinese Scholarship Council (CSC) (CSC ID: 201206340004, to Z.Z.).

Acknowledgements

We would like to thank the staff of the Laboratory of Endocrinology for measuring serum thyroid hormones.

References

- Bianco AC, Salvatore D, Gereben B, Berry MJ & Larsen PR 2002 Biochemistry, cellular and molecular biology, and physiological roles of the iodothyronine selenodeiodinases. *Endocrine Reviews* **23** 38–89. (doi:10.1210/edrv.23.1.0455)

- Dellovade TL, Zhu YS & Pfaff DW 1999 Thyroid hormones and estrogen affect oxytocin gene expression in hypothalamic neurons. *Journal of Neuroendocrinology* **11** 1–10. (doi:10.1159/000054473)
- Dentice M & Salvatore D 2011 Deiodinases: the balance of thyroid hormone: local impact of thyroid hormone inactivation. *Journal of Endocrinology* **209** 273–282. (doi:10.1530/JOE-11-0002)
- Dyess EM, Segerson TP, Liposits Z, Paull WK, Kaplan MM, Wu P, Jackson IM & Lechan RM 1988 Triiodothyronine exerts direct cell-specific regulation of thyrotropin-releasing hormone gene expression in the hypothalamic paraventricular nucleus. *Endocrinology* **123** 2291–2297. (doi:10.1210/endo-123-5-2291)
- Fekete C & Lechan RM 2014 Central regulation of hypothalamic-pituitary-thyroid axis under physiological and pathophysiological conditions. *Endocrine Reviews* **35** 159–194. (doi:10.2340/16501977-2050)
- Fliers E, Kalsbeek A & Boelen A 2014 Beyond the fixed setpoint of the hypothalamus-pituitary-thyroid axis. *European Journal of Endocrinology* **171** R197–R208. (doi:10.1530/EJE-14-0285)
- Fliers E, Klieverik LP & Kalsbeek A 2010 Novel neural pathways for metabolic effects of thyroid hormone. *Trends in Endocrinology & Metabolism* **21** 230–236. (doi:10.1016/j.tem.2009.11.008)
- Herwig A, Campbell G, Mayer CD, Boelen A, Anderson RA, Ross AW, Mercer JG & Barrett P 2014 A thyroid hormone challenge in hypothyroid rats identifies T3 regulated genes in the hypothalamus and in models with altered energy balance and glucose homeostasis. *Thyroid* **24** 1575–1593. (doi:10.1089/thy.2014.0169)
- Klieverik LP, Janssen SF, van Riel A, Foppen E, Bisschop PH, Serlie MJ, Boelen A, Ackermans MT, Sauerwein HP, Fliers E *et al.* 2009 Thyroid hormone modulates glucose production via a sympathetic pathway from the hypothalamic paraventricular nucleus to the liver. *PNAS* **106** 5966–5971. (doi:10.1073/pnas.0805355106)
- Klieverik LP, Sauerwein HP, Ackermans MT, Boelen A, Kalsbeek A & Fliers E 2008 Effects of thyrotoxicosis and selective hepatic autonomic denervation on hepatic glucose metabolism in rats. *American Journal of Physiology: Endocrinology and Metabolism* **294** E513–E520. (doi:10.1073/pnas.0805355106)
- Li WW, Le Goascogne C, Ramaugue M, Schumacher M, Pierre M & Courtin F 2001 Induction of type 3 iodothyronine deiodinase by nerve injury in the rat peripheral nervous system. *Endocrinology* **142** 5190–5197. (doi:10.1210/endo.142.12.8532)
- Lopez M, Alvarez CV, Nogueiras R & Dieguez C 2013 Energy balance regulation by thyroid hormones at central level. *Trends in Endocrinology & Metabolism* **19** 418–427. (doi:10.1016/j.beem.2014.04.004)
- Lopez M, Varela L, Vazquez MJ, Rodriguez-Cuenca S, Gonzalez CR, Velagapudi VR, Morgan DA, Schoenmakers E, Agassandian K, Lage R *et al.* 2010 Hypothalamic AMPK and fatty acid metabolism mediate thyroid regulation of energy balance. *Nature Medicine* **16** 1001–1008.
- Magner JA 1990 Thyroid-stimulating hormone: biosynthesis, cell biology, and bioactivity. *Endocrine Reviews* **11** 354–385. (doi:10.1210/edrv-11-2-354)
- Mittag J, Lyons DJ, Sallstrom J, Vujovic M, Dudazy-Gralla S, Warner A, Wallis K, Alkemade A, Nordstrom K, Monyer H *et al.* 2013 Thyroid hormone is required for hypothalamic neurons regulating cardiovascular functions. *Journal of Clinical Investigation* **123** 509–516. (doi:10.1172/JCI65252)
- Mullur R, Liu YY & Brent GA 2014 Thyroid hormone regulation of metabolism. *Physiological Reviews* **94** 355–382. (doi:10.1152/physrev.00030.2013)
- Murphy M, Jethwa PH, Warner A, Barrett P, Nilaweera KN, Brameld JM & Ebling FJ 2012 Effects of manipulating hypothalamic triiodothyronine concentrations on seasonal body weight and torpor cycles in Siberian hamsters. *Endocrinology* **153** 101–112. (doi:10.1210/en.2011-1249)
- Palkovits M 1973 Isolated removal of hypothalamic or other brain nuclei of the rat. *Brain Research* **59** 449–450. (doi:10.1016/0006-8993(73)90290-4)
- Persani L & Bonomi M 2014 Chapter 27 – Uncertainties in endocrine substitution therapy for central endocrine insufficiencies: hypothyroidism. In *Handbook of Clinical Neurology*, pp 397–405. Eds MK Eric Fliers & AR Johannes. Philadelphia, PA, USA: Elsevier. (doi:10.1016/B978-0-444-59602-4.00027-7)
- Potter GB, Zarach JM, Sisk JM & Thompson CC 2002 The thyroid hormone-regulated corepressor hairless associates with histone deacetylases in neonatal rat brain. *Molecular Endocrinology* **16** 2547–2560. (doi:10.1210/me.2002-0115)
- Roselli CE, Jorgensen EZ, Doyle MW & Ronnekleiv OK 1997 Expression of the orphan receptor steroidogenic factor-1 mRNA in the rat medial basal hypothalamus. *Molecular Brain Research* **44** 66–72. (doi:10.1016/S0169-328X(96)00187-8)
- Segerson TP, Kauer J, Wolfe HC, Mobtaker H, Wu P, Jackson IM & Lechan RM 1987 Thyroid hormone regulates TRH biosynthesis in the paraventricular nucleus of the rat hypothalamus. *Science* **238** 78–80. (doi:10.1126/science.3116669)
- Sinha RA, Singh BK & Yen PM 2014 Thyroid hormone regulation of hepatic lipid and carbohydrate metabolism. *Trends in Endocrinology & Metabolism* **25** 538–545. (doi:10.1016/j.tem.2014.07.001)
- Sjogren M, Alkemade A, Mittag J, Nordstrom K, Katz A, Rozell B, Westerblad H, Arner A & Vennstrom B 2007 Hypermetabolism in mice caused by the central action of an unliganded thyroid hormone receptor alpha1. *EMBO Journal* **26** 4535–4545. (doi:10.1038/sj.emboj.7601882)
- Swanson LW & Sawchenko PE 1983 Hypothalamic integration: organization of the paraventricular and supraoptic nuclei. *Annual Review of Neuroscience* **6** 269–324. (doi:10.1146/annurev.ne.06.030183.001413)
- Tu HM, Legradi G, Bartha T, Salvatore D, Lechan RM & Larsen PR 1999 Regional expression of the type 3 iodothyronine deiodinase messenger ribonucleic acid in the rat central nervous system and its regulation by thyroid hormone. *Endocrinology* **140** 784–790. (doi:10.1210/endo.140.2.6486)
- Warner A & Mittag J 2012 Thyroid hormone and the central control of homeostasis. *Journal of Molecular Endocrinology* **49** R29–R35. (doi:10.1530/JME-12-0068)
- Wiersinga WM & Chopra IJ 1982 Radioimmunoassay of thyroxine (T₄), 3,5,3'-triiodothyronine (T₃), 3,3',5'-triiodothyronine (reverse T₃, rT₃), and 3,3'-diiodothyronine (T₂). *Methods in Enzymology* **84** 272–303.

Received in final form 27 January 2016

Accepted 10 February 2016

Accepted Preprint published online 10 February 2016