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Research Report

Effects of acute microinjections of thyroid hormone to the preoptic region of hypothyroid adult male rats on sleep, motor activity and body temperature ☆

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ABSTRACT

Thyroid hormones induce short-latency nongenomic effects in adult brain tissue, suggesting that their acute administration would affect brain activity in intact animals. The influence on EEG-defined sleep of acute restoration of L-3,3′5-triiodothyronine (T_3) to a sleep-regulatory brain region, the preoptic region, was examined in hypothyroid rats. Sleep parameters were monitored for 48 h weekly: for 24 h immediately following a control microinjection and for an additional 24 h after a second microinjection including a T_3 dose to the preoptic region or lateral ventricle. Male albino rats were implanted with EEG and EMG electrodes, abdominal temperature/activity transponders and unilateral lateral ventricle cannulae or bilateral preoptic region cannulae, and were given 0.02% *n*-propythiouracil (PTU) in their drinking water for 4 weeks. For histologically-confirmed bilateral preoptic region cannula placements ($N=7$), effects of T_3 (especially a 3 μg dose) were apparent within 10 h of injection as decreases in REM, NREM and total sleep and increases in waking and activity. Minimal effects of lateral ventricle T_3 microinjection were demonstrated ($N=5$). Significant effects due to the time of day on the experimental measures were seen in both lateral ventricle and preoptic region groups, but these effects did not interact with the effect of administered hormone dose. These effects of T_3 microinjection to the preoptic region were demonstrated after acute injections and within hours of injection rather than after chronic administration over days.

Abbreviations: Anterior-posterior, AP; dorsal-ventral, DV; medial preoptic area, MPOA; electromyographic, EMG; medial-lateral ML; median preoptic nucleus, MePO; phosphate buffered saline, PBS; *n*-propythiouracil, PTU; L-3,3′5-triiodothyronine, T_3; L-thyroxine, T_4; wakefulness, W

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1. Introduction

A primary clinical feature of adult dysthyroidism is disturbed sleep (Demet et al., 2002; Emerson et al., 1987; Gunnarsson et al., 2001; Kales et al., 1967; Orgiazzi and Mornex, 1990; Sridhar and Madhu, 1996; Watt et al., 2006; Whybrow and Bauer, 2005a, 2005b). Insomnia is a common symptom in patients with hyperthyroidism (Demet et al., 2002; Orgiazzi and Mornex, 1990; Whybrow and Bauer, 2005b). Hypersomnolence and drowsiness are frequent presenting conditions in hypothyroidism (Emerson et al., 1987; Gunnarsson et al., 2001; Sridhar and Madhu, 1996; Whybrow and Bauer, 2005a), and have been attributed to poor nighttime sleep (Kales et al., 1967). Treatments which restore the patients to euthyroid hormone levels also mitigate many of the sleep-related complaints (Gunnarsson et al., 2001; Kales et al., 1967; Watt et al., 2006; Whybrow and Bauer, 2005a). While electroencephalographic (EEG) studies have generally confirmed altered sleep patterns during thyroid disturbance in humans (Dunleavy et al., 1974; Kales et al., 1967) and after chronic experimental manipulation of thyroid function in animals (Carpenter and Timiras, 1982; Gull et al., 1989; Salin-Pascual et al., 1997), there are inconsistencies in the results (Eastman and Rechtschaffen, 1979; Joffe et al., 1995; Salin-Pascual et al., 1997). A potential link between sleep mechanisms and thyroid function is also suggested by the drop in thyroid hormone levels consistently observed with chronic sleep deprivation (Bergmann et al., 1989, 1995; Bernal et al., 2003; Everson and Reed, 1995; Everson and Crowley, 2004; Patel et al., 2011).

Over the last 15 years, a subset of the actions of thyroid hormones has been firmly established as occurring at the plasma membrane or within cytoplasm (Axelband et al., 2011; Cheng et al., 2010; Davis et al., 2011; Dudley and Baumgarten, 1993; Leonard and Farwell, 1997). While signaling pathways outside of the nucleus may also, of course, subsequently influence gene expression, the term "nongenomic" (Axelband et al., 2011; Cheng et al., 2010; Davis et al., 2011) has been widely used to distinguish such mechanisms from the classical interactions of thyroid hormones with the heterodimeric thyroid hormone nuclear receptor (TR) (Oppenheimer et al., 1972; Weinberger et al., 1986). In neonatal brain, the mostly irreversible developmental effects of thyroid hormones are usually ascribed to TR-related gene regulation (Bernal et al., 2003; Patel et al., 2011), although it is becoming clear that nongenomic effects of the hormones also play a role in brain maturation (Scapin et al., 2010). In the mature brain, TR-mediated effects are less ubiquitous than in the neonatal brain (Galeeva et al., 2002; Haas et al., 2004) and there are at least two distinct membrane binding sites for L-3,3′5-triiodothyronine (T_3) in nerve terminal preparations (synaptosomes) from adult CNS (Mashio et al., 1982, 1983; Sarkar and Ray, 1998). Thyroid hormones have nongenomic effects in mature nervous systems through a variety of mechanisms (Alzoubi et al., 2005; Desouza et al., 2011; Gerges et al., 2001; Sarkar et al., 2011) including an influence at neurotransmitter receptors (Caria et al., 2009; Chapell et al., 1998; Losi et al., 2008; Martin et al., 1996, 2004; Puia and Losi, 2011; Sarkar et al., 2006). T_3 has predominantly inhibitory effects on $GABA_A$ receptor function in a variety of experimental systems (Chapell et al., 1998; Martin et al., 1996) (see (Wiens and Trudeau, 2006) for review).

Thyroid hormones are rapidly taken up into adult brain and concentrated in synaptosomal fractions (Dratman et al., 1976, 1987; Dratman and Crutchfield, 1978). L-thyroxine (T_4) secreted from the thyroid gland is deiodinated by brain iodothyronine deiodinases, 5'D-II and 5D-III (Dratman and Crutchfield, 1978; Dratman et al., 1983; Guerrero et al., 1988; Tanaka et al., 1981). Levels of thyroid hormones in the adult CNS resist hyperthyroidism and hypothyroidism, suggesting homeostatic mechanisms to maintain the concentrations of the hormones in neural tissue (Dratman et al., 1983; Kundu et al., 2006; Sarkar and Ray, 1994). Patterns of circadian variations in levels of T_3 in brain vary by anatomical region and contrast with those in serum (Campos-Barros et al., 1997). Thyroid hormones may therefore exert signaling functions in adult brain distinct from systemic effects of the hormones. Somewhat analogously, neurosteroids are synthesized *de novo* from cholesterol in brain tissue and vary independently of circulating steroid levels (see (Baulieu, 1997)).

The results of previous studies suggest that thyroid hormones are likely to have some nongenomic effects on sleep, which would be apparent within hours of an acute injection instead of after days of chronic administration, and that the preoptic region would be a likely brain injection site to elicit these effects.

The study was performed in order to confirm the hypothetical effects of acute T_3 administration to the preoptic region on sleep. We administered T_3 acutely so as to investigate the effects of the hormone which are not due to chronic influences on gene expression.

2. Results

2.1. *Effects of T_3 administered to the preoptic region*

After histologically-confirmed injections to the preoptic region were identified (Fig. 1), EEG-defined states of consciousness

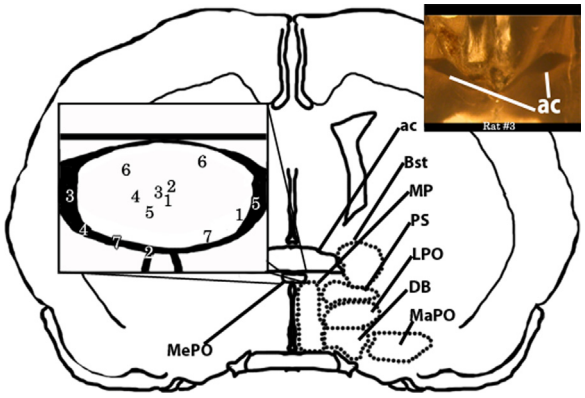

Fig. 1 – Seven rats were histologically confirmed to have cannula tips within the preoptic region and centered in the median preoptic nucleus (MePO). Each number indicates the two cannulae terminae. The inset shows a section from Rat 3. Other structures are indicated by the abbreviations ac (anterior commissure), Bst (bed nucleus of the stria terminalis, MP (medial preoptic area), PS (parastrial nucleus), LPO (lateral preoptic area), DB (diagonal band of Broca), MaPo (magnocellular preoptic nucleus).