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Research Report

Effects of acute microinjections of thyroid hormone to the preoptic region of euthyroid adult male rats on sleep and motor activity

Joseph V. Martin[a,b,*], *Phillip F. Giannopoulos*[a], *Steven X. Moffett*[b], *Thomas D. James*[b]

[a]Biology Department, Rutgers University, 315 Penn Street, Camden, NJ 08102, USA
[b]Center for Computational and Integrative Biology, Rutgers University, 315 Penn Street, Camden, NJ 08102, USA

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ABSTRACT

In adult brain tissue, thyroid hormones are known to have multiple effects which are not mediated by chronic influences of the hormones on heterodimeric thyroid hormone nuclear receptors. Previous work has shown that acute microinjections of L-triiodothyronine (T3) to the preoptic region significantly influence EEG-defined sleep in hypothyroid rats. The current study examined the effects of similar microinjections in euthyroid rats. In 7 rats with histologically confirmed microinjection sites bilaterally placed in the preoptic region, slow-wave sleep time was significantly decreased, but REM and waking were increased as compared to vehicle-injected controls. The EEG-defined parameters were significantly influenced by the microinjections in a biphasic dose-response relationship; the lowest (0.3 μg) and highest (10 μg) doses tested were without significant effect while intermediate doses (1 and 3 μg) induced significant differences from controls. There were significant diurnal variations in the measures, yet no significant interactions between the effect of hormone and time of day were demonstrated. Core body temperature was not significantly altered in the current study. The demonstration of effects of T3 within hours instead of days is consistent with a rapid mechanism of action such as a direct influence on neurotransmission. Since the T3-mediated effects were robust in the current work, euthyroid rats retain thyroid hormone sensitivity which would be needed if sleep-regulatory mechanisms in the preoptic region are continuously modulated by the hormones.

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Abbreviations: AP, anterior-posterior; PR, preoptic region; DV, dorsal-ventral; EEG, electroencephalography; EMG, electromyography; ML, medial-lateral; MPA, medial preoptic area; MePO, median preoptic nucleus; T3, L-triiodothyronine; T4, L-thyroxine; TR, nuclear thyroid hormone receptors; vlPOA, ventrolateral preoptic area

*Corresponding author at: Center for Computational and Integrative Biology, 315 Penn Street, Rutgers University, Camden, NJ 08102, USA. Fax: +1856 225 6312.

E-mail addresses: joe.martin@rutgers.edu (J.V. Martin), philg82@gmail.com (P.F. Giannopoulos), smoffett@camden.rutgers.edu (S.X. Moffett), tomjames@camden.rutgers.edu (T.D. James).

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1. Introduction

Neurological symptoms of thyroid dysfunction, such as anxiety and disturbed sleep, have been frequently reported in clinical studies (Demet et al., 2002; Emerson et al., 1987; Watt et al., 2006; Whybrow and Bauer, 2005). However, conflicting results of laboratory studies of EEG-defined sleep have been reported, perhaps due to differences in the methods used for inducing chronic hypothyroidism or hyperthyroidism (Browning et al., 1954; Carpenter and Timiras, 1982; Dunleavy et al., 1974; Eastman and Rechtschaffen, 1979; Hemmeter et al., 1998; Hermann and Quarton, 1964; Kales et al., 1967; Salin-Pascual et al., 1997; Watt et al., 2006; Whybrow and Bauer, 2005). A weak effect on sleep EEG has been shown in the shorter term through pulsatile intravenous injections of thyrotropin-releasing hormone in humans (Hemmeter et al., 1998), but no investigations have been performed with direct injections of thyrotropin-releasing hormone or thyroid hormone (TH) to brain tissue. Recently, we have shown an inhibition of slow-wave sleep (SWS) following single-injection administration of TH to the preoptic region (PR) of hypothyroid rats (Moffett et al., in review). Therefore, the regulation of sleep by thyroid hormones might, at least in part, be due to relatively short-term activities (over hours instead of days). Such activities might be due to mechanisms other than the well-known and relatively gradual regulation of gene expression by the hormones.

Whereas previous studies had focused on effects of chronic alterations in TH levels which would be consistent with an action on a mechanism involving long-term changes in gene expression, the current study continued to explore the effects of single injections of TH, following the protocol of Moffett et al. (in review). TH can alter neural processes and excitability without binding nuclear thyroid hormone receptors (TR) to directly alter gene transcription, particularly in the adult brain. Several such nongenomic mechanisms have been characterized recently. Specifically, both 3,3′5-triiodothyronine (T3) and thyroxine (T4) non-competitively inhibit native $GABA_A$ receptors in preparations of pre- and post-synaptic brain membranes (synaptoneurosomes) (Martin et al., 1996, 2004). Recombinant $GABA_A$ receptors expressed in human embryonic kidney cells (HEK-293) and *Xenopus* oocytes are inhibited by thyroid hormone, while at high concentrations, thyroid hormone directly gates recombinant $GABA_A$ receptors (Chapell et al., 1998). Additionally, thyroid hormones alter protein phosphorylation in a biphasic, dose-dependent manner in a nerve terminal (synaptosomal) lysate (Sarkar et al., 2006, 2011) and inhibit synaptosomal Na^+/K^+ATPase (Sarkar and Ray, 1993). Molecular modeling shows that T3 has similar molecular dimensions to the neurosteroid pregnenolone sulfate (Martin et al., 1996), which has been shown to alter EEG in rats (Lancel et al., 1994). Pregnenolone has also been shown to alter EEG in humans (Steiger et al., 1993). Within the CNS, T3 has additional neurosteroid-like characteristics, such as local synthesis from precursor molecules (T4) and effects on $GABA_A$ receptors (Belelli and Lambert, 2005; Dratman, 1974; Gereben et al., 2008; Kohrle et al., 1987; Martin et al., 1996; Martin et al., 2004; Puia and Losi, 2011). Furthermore, thyroid hormone influences the noradrenergic system in brain tissue, suggesting a potential role of thyroid hormone as a noradrenergic cotransmitter (Dratman, 1974; Dratman and Gordon, 1996). Thyroid hormone is localized in adrenergic systems and axonally transported to noradrenergic projection sites (Gordon et al., 1999; Rozanov and Dratman, 1996). Tyrosine hydroxylase activity and norepinephrine synthesis are regulated by T4, while conversion of T4 to T3 by 5′deiodinase is decreased by injection of the α- and β-adrenergic blockers prazosin and propranolol (Dratman, 1974; Dratman and Gordon, 1996; Emlen et al., 1972; Gordon et al., 1999; Prange et al., 1970; Rozanov and Dratman, 1996). Together, these investigations indicate that thyroid hormones can regulate neuronal activity through numerous potentially nongenomic mechanisms and raise the possibility that the hormones may have physiologically-relevant influences on adult brain f unction, resembling neurosteroids, neurotransmitters or cotransmitters.

The PR, includes numerous brain regions involved in sleep regulation (McGinty and Szymusiak, 2001). The medial preoptic area (MPA) is sensitive to microinjections of the highly potent benzodiazepine triazolam, an effect which is inhibited by flumazenil and calcium channel blockers (Martin and Mendelson, 1990; Mendelson et al., 1989; Mendelson and Martin, 1992). The median preoptic nucleus (MePO) and ventrolateral preoptic area (vlPOA) influence sleep behavior by regulating the activity of sleep-related brain nuclei (McGinty and Szymusiak, 2003; Saper et al., 2001). Both c-FOS and electrophysiological data show GABAergic neurons of the MePO and vlPOA are active just before and during SWS and REM (Gong et al., 2000; Gong et al., 2004; Suntsova et al., 2002). GABAergic inhibition by the MePO and vlPOA of ascending arousal systems, such as the dorsal raphe nuclei, locus coeruleus, and the orexinergic lateral hypothalamic area, results in the transition to and maintenance of sleep (Chou et al., 2002; Uschakov et al., 2006, 2007; Yoshida et al., 2006; Zardetto-Smith and Johnson, 1995). In our previous work (Moffett et al., in review), the administration of T3 to the PR was effective in transiently reducing slow-wave sleep in hypothyroid rats, an effect which mimics the inhibitory influence of noradrenaline on the sleep-promoting MePO (McGinty and Szymusiak, 2003; Saper et al., 2001).

In the previous work, the rats were made hypothyroid so as to examine the effects of thyroid hormone injection against a lowered background of hormone. The finding of single-injection effects (within hours instead of days) of T3 in that study raises the question of whether the effects of T3 noted were due to a restoration of depleted T3 levels in the PR, or whether an effect might also be elicited by a transient elevation of T3 above normal levels in euthyroid rats. If a nongenomic mechanism, such as a membrane effect, mediates continuous thyroid hormone regulation of sleep, then we would hypothesize that the receptor mediating such a response should not be maximally stimulated under euthyroid conditions, so as to remain responsive to changes in ambient levels of the hormone. In the current study, injections of T3 to the PR of rats with normal thyroid state were performed and data were taken over a shorter term than would be done to observe genomic effects.