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Research report

Levothyroxin replacement therapy restores hypothyroidism induced impairment of L-LTP induction: Critical role of CREB

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ABSTRACT

Cyclic-AMP response element binding protein (CREB) is a transcription factor crucial for late phase long-term potentiation (L-LTP) induction and maintenance. Upon multiple high frequency stimulation (MHFS), large Ca^{2+} influx activates adenylyl cyclase. This, in turn, activates PKA, which by itself or through MAPK p42/p44 can activate (phosphorylate) CREB. Upon phosphorylation, P-CREB activates multiple genes essential for L-LTP generation. Calcium calmodulin kinase IV (CaMKIV) is also activated by calcium and can directly activate CREB. We have shown previously that hypothyroidism impairs L-LTP and reduces the basal protein levels of CREB, MAPK p42/p44, and CaMKIV in area CA1 of the hippocampus. In the present study, levels of these signaling molecules were determined in area CA1 during the induction and maintenance phases of L-LTP. Standard MHFS was used to evoke L-LTP in the CA1 area of hypothyroid, levothyroxin treated hypothyroid and sham control anesthetized adult rats. Chronic levothyroxin treatment reversed hypothyroidism-induced L-LTP impairment. Five minutes after MHFS, western blotting showed an increase in the levels of P-CREB, and P-MAPK p42/p44 in sham-operated control, and levothyroxin treated hypothyroid animals, but not in hypothyroid animals. The protein levels of total CREB, total MAPK p42/p44, BDNF and CaMKIV were not altered in all groups five minutes after MHFS. Four hours after MHFS, the levels of P-CREB, and P-MAPK p42/p44 remained unchanged in hypothyroid animals, while they were elevated in sham-operated control, and levothyroxin treated hypothyroid animals. We conclude that respective normalized phosphorylation of essential kinases such as P-CREB and P-MAPK p42/p44 is correlated with restoration of normal L-LTP induction and maintenance in the CA1 area of levothyroxin-treated hypothyroid animals.

1. Introduction

Adult-onset hypothyroidism may cause several dysfunctions in the CNS (Burmeister et al., 2001; Koromilas et al., 2010) including learning and memory impairments (Gerges et al., 2004b; Alzoubi et al., 2006b, 2009; Reid et al., 2007; Rivas and Naranjo, 2007; He et al., 2011; Ge et al., 2012). Patients with untreated hypothyroidism have consistently reported symptoms of severe cognitive impairments including inability to concentrate, slow mentation, poor memory for recent events and inability to calculate and understand complex questions (Haggerty et al., 1990; Osterweil et al., 1992; Mennemeier et al., 1993; Leentjens and Kappers, 1995; Burmeister et al., 2001; Samuels et al., 2007a; Aghili et al., 2012; Yin et al., 2013). Earlier studies of animal models of adult onset hypothyroidism reported severe impairment of learning and memory; coupled with impairment of both early (E-) and late (L-) phase long-term potentiation (LTP) (Gerges et al., 2004b; Alzoubi et al., 2005, 2006b, 2009; Tong et al., 2007). These studies also established some possible molecular deficits that may be responsible for this impairment (Gerges and Alkadhi, 2004; Alzoubi et al., 2005, 2009).

Levothyroxin (thyroxin) replacement therapy improves memory in subclinical hypothyroid subjects (Osterweil et al., 1992; Monzani et al., 1993; Baldini et al., 1997; Jensovsky et al., 2002). However, clinical reports show variable results as to whether the thyroid hormone replacement therapy fully restores the hypothyroidism-induced impaired learning and memory (Treadway et al., 1967; Jaeschke et al., 1996; Capet et al., 2000; Miller et al., 2006; but see Mennemeier et al., 1993; Leentjens and Kappers, 1995; Wekking et al., 2005; Samuels et al., 2007b). While a large body of literature is available on the effect of thyroid hormone deficiency during the developmental stage (e.g. Reid et al., 2007), adult onset hypothyroidism has not been adequately studied. We have shown previously that thyroxin replacement therapy reverses hypothyroidism induced impairment of both E-LTP (Alzoubi et al., 2005), and L-LTP (Alzoubi et al., 2009).

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Long-term potentiation (LTP) is an activity-dependent enhancement of synaptic transmission widely accepted as a cellular model for learning and memory (Bliss and Collingridge, 1993; Barnes, 1995). The two distinct forms of N-methyl-D-aspartate (NMDA)-dependent hippocampal E-LTP and L-LTP differ from each other in number of facets. The E-LTP, a marked potentiation of the synaptic responses lasting 30 min to 3 h, can be induced by one train of stimuli at 100 Hz applied for a period of 1 s. In contrast, L-LTP requires four trains of stimuli and lasts more than 3 h (Frey et al., 1993b; Huang et al., 1994). It is well recognized that protein kinases are essential intermediates in the induction and/or maintenance of LTP and memory. While E-LTP mainly depends on phosphorylation of existing CaMKII molecules (Malenka et al., 1989; Pettit et al., 1994; Thomas et al., 1994; Lledo et al., 1995; Giese et al., 1998; Roberts et al., 1998), L-LTP depends on new protein synthesis through kinases-induced activation of transcription factors such as CREB. These kinases include PKA (Abel et al., 1997), MAPK (English and Sweatt, 1997; Impey et al., 1998; Huang et al., 2000) and CAMKIV (Bito et al., 1996; Ho et al., 2000; Kang et al., 2001). Upon multiple high frequency stimulation (MHFS), large and highly localized Ca^{2+} influx activates adenylyl cyclase type I (Bach et al., 1999; Wong et al., 1999; Poser and Storm, 2001; Ferguson and Storm, 2004). This in turn activates PKA, which by itself or through MAPKp44/42 (ERK1/2) can activate (phosphorylate) CREB (Impey et al., 1998; Huang et al., 2000; Rosenblum et al., 2002). Upon phosphorylation, P-CREB activates multiple genes essential for L-LTP expression (Kandel, 2001; Barco et al., 2002, 2003; Alarcon et al., 2004). CaMKIV is also activated by calcium and can directly activate CREB (Bito et al., 1996; Tokuda et al., 1997; Kang et al., 2001; Kasahara et al., 2001). Active (phospho) CREB can be deactivated via protein phosphatases including calcineurin (Bito et al., 1996). We have shown previously that levothyroxin restores hypothyroidism impairment of L-LTP and reduction in the basal protein levels of CREB, ERK1/2, and CaMKIV during L-LTP maintenance phase in area CA1 of the hippocampus (Alzoubi et al., 2009). To further elucidate molecular changes during L-LTP, in the present study the levels of these signaling molecules were determined in area CA1 of the hippocampus at the induction and maintenance phases of L-LTP during adult-onset hypothyroidism and after levothyroxin replacement therapy.

2. Materials and methods

All animal experiments were carried out in accordance with the NIH Guide for Care and Use of Laboratory Animals and approved by the University of Houston's Institutional Animal Care and Use Committee. Adult male Wistar rats (Harlan, Indianapolis, IN) weighing 230–280 g at arrival, were housed on a 12:12 h light/dark schedule (lights on at 7 A.M.) in Plexiglas cages (6 rats per cage) at 25 °C with *ad libitum* access to standard rodent chow and water. After arrival at the research facility, all rats were allowed one week to acclimate before manipulations began.

2.1. Thyroidectomy

Rats were anesthetized with an i.p. injection of a mixture of ketamine (100 mg/kg), xylazine (2.5 mg/kg) and acepromazine (2.5 mg/kg). Standard procedures were used for thyroidectomy as described (Alzoubi et al., 2007; Alzoubi and Alkadhi, 2007). Briefly, a midline skin incision was made along the length of the neck. The underlying tissues were cleared and the salivary glands were retracted laterally. The two halves of the sternohyoid muscle were separated and retracted laterally. Thyroid muscle was separated from the thyroid gland lobes and retracted along with the sternohyoid muscle. A midline cut was made in the isthmus and the thyroid glands were excised bilaterally. Extreme care was taken so as not to damage the laryngeal nerve. Sham (euthyroid/control) animals underwent the same surgical procedures without removal of the thyroid gland. Rats were monitored closely for at least one week after surgery for possible complications and animals were used for experimentation 4 weeks later.

2.2. Measurement of thyroxin (T4) and thyroid stimulating hormone (TSH) levels

Blood samples were collected from animals in all groups, just before dissection of the hippocampus, and immediately centrifuged at 14,000 rpm for 15 min. The serum was quickly frozen at −80 °C until assayed by a radioimmunoassay kit (ICN Pharmaceuticals, Orangeburg, NY) to measure total serum T4 and to determine TSH in the serum with Rat Thyroid Stimulating Hormone (rTSH), BiotraK, enzyme immunoassay system (Amersham Biosciences, NJ). Hormones were measured in 50 μl aliquots. All samples were done in triplicate.

2.3. Treatments

All treatments were started 4 weeks after thyroidectomy. Separate sets of animal groups were used for each of the two time points (5 min and 4 h after MHFS). The treatment groups are control (sham operated), thyroidectomized (hypothyroid), and thyroxin-treated thyroidectomized. Thyroxin-treated thyroidectomized group was administered levothyroxin (Sigma, St. Louis, MO) 20 μg/kg/day i.p. for at least 4 weeks, until electrophysiological recording was done. On each experimental day, thyroxin treatment was administered 2 h before starting the experimental procedure. Control and thyroidectomized groups received the same volume of saline (0.9%, w/v NaCl) i.p. for the same period.

2.4. Induction of L-LTP

Recording of field excitatory postsynaptic potential (fEPSPs) from CA1 area of the hippocampus of anesthetized (urethane 1.2 g/kg) rats was performed as described (Alzoubi et al., 2007; Alzoubi and Alkadhi, 2007). Briefly, a rat was placed in a stereotaxic frame and holes were drilled in appropriate regions of the skull. A concentric bipolar stimulating electrode was placed in the CA3 region of the left hippocampus (AP, 2.3; L, 3.5; D, 2.8) for stimulation of the CA3 region and a glass capillary recording electrode (1–5 ΩM), filled with 1% Fast Green in 2 M NaCl, was placed in the CA1 of the right hippocampus (AP, 2.3; L, 1.8; D, 2.0/3.0) to record the field potential response. Fast green was used to mark the position of the recording electrode (by dissection after conclusion of the experiment). Stimulus intensity, approximately 30% of the maximum response, was chosen (by constructing input/output curves) to evoke test responses every 30 s at 0.04 Hz throughout the experiment. L-LTP was evoked by multiple high frequency stimulation (MHFS) consisted of 4 trains given at 2.5-min intervals. Each train consisted of 8 pulses (400 Hz) repeated every 10 s for a period of 30 s (Gerges and Alkadhi, 2004; Alzoubi et al., 2006b).

2.5. Hippocampus dissection

Animals were sacrificed and processed as described (Alzoubi et al., 2006a, 2008; Alzoubi and Alkadhi, 2007). The CA1 areas of the hippocampus were immediately dissected out. The right hippocampi were removed to dissect out the dorsal (septal) and the ventral (temporal) parts of area CA1 (Gerges et al., 2004a, 2005; Alzoubi et al., 2005, 2009; Aleisa et al., 2006). The hippocampus was immediately placed on a filter paper soaked in ice-cold 0.2 M sucrose (to prevent brain sticking to the filter paper) over a covered petri dish filled with dry ice. The two tips of the hippocampus were