In Vitro Actions of Thyroid Hormone on Protein Phosphorylation in a Nucleus-free Subcellular Fraction from Adult Rat Brain.

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Introduction

Several converging lines of evidence point to a unique mechanism of action of thyroid hormones (TH) in adult brain. As revealed by debilitating neurological signs and symptoms associated with thyroid dysfunction in adults (Lauterberg, 1990; Unger, 1987), TH play fundamental roles in the central nervous system. In contrast to the neurological disorders related to development abnormalities in TH, most of the ONH signs and symptoms of adult-onset thyroid dysfunction are reversible with proper adjustment of the circulating TH (Grihaut and Gordon, 1986; Hayashi and Kordower, 1997; Buijs and Prange, 2002; Sarkar 2002). In developing nervous system, the major effect of thyroid hormones is generally considered to be the result of stimulation of a nuclear receptor which is also found in adult peripheral tissues (Opperhöfer et al., 1998). High levels of 3,5,3'-L-triiodothyronine (T3) have been found in nerve terminals from adult brain, suggesting a synaptic action of TH (Dratman, 1974; Mason, et al., 1993; Sarkar and Ray, 1984; Dratman and Gordon, 1986).

In recent years, the possibility of direct nongenomic actions of TH in certain types of cells, including mature neurons, is gaining much attention (Martin et al., 1990; Sarkar and Ray, 2003; see Dratman and Gordon, 1996; Sarkar 2002). However, the mechanisms of action of TH in the regulation of synaptic functions in adult neurons are poorly understood. The present study examines the possibility of a nongenomic action of T3 to modulate protein phosphorylation in synaptosomes from adult rat brain.

Methods

Animals

Young adult male Sprague-Dawley rats (250-300 g) were decapitated and their brains immediately dissected in ice-cold isotonic saline.

Preparation of Synaptosomes

Synaptosomes were purified from cerebral cortical tissue using the method of Hajós (1976). A 3% pellet was prepared by differential centrifugation in 0.9 M sucrose and layered on 0.5 M sucrose. Subsequently an additional centrifugation at 3,000 g for 30 min. The G M layer was diluted to 0.4 M. The pellet sedimenting after 20 min at 12,000 g (purified synaptosomes) was lysed with hypotonic shock and used for the incubations.

Incubation

The resulting preparation of soluble and membrane protein was incubated in 10 mM MgCl₂, 0.1 mM EDTA, and 50 mM HEPES (pH 7.4) with various concentrations of 3,5,3'-L-triiodothyronine (T3) and 3 µg of γ-[32P]-ATP for varying times at 37°C. The reaction was terminated by addition of SDS-Sample buffer (Laemmli, 1970).

SOS-PAGE and Autoradiography

The samples were denatured for 3 minutes at 95°C, subjected to electrophoresis on 7.5% SDS-PAGE at constant current, and stained with silver nitrate. The dried gels were then exposed to x-ray film at -40°C for 48 hours and the optical densities of bands in the gel and the resulting autoradiograph were quantified using the ONE-DISCAN program (ScancoMet, Inc., Faribault, VA).

References


Summary and Conclusion

(1) A dramatic enhancement of incorporation of [32P]P into unit amount of protein was demonstrated in three proteins of molecular weights 79, 96, and 79 kDa, following incubation with concentrations of T3 from 10-100 mM. Physiological concentrations of T3 in nerve terminals are ~15 nM (Martin et al., 1990; Sarkar and Ray, 1994). The degree of phosphorylation of the 96 and 79 kDa proteins stimulated by these concentrations of T3 was higher than the 114 kDa protein. However, the higher doses of T3 (300-1000 nM) showed a lesser stimulation of phosphorylation of all three proteins, indicating a biphasic action. Overall, T3 induced varying levels of phosphorylation of several proteins in adult rat brain and cerebrocortical synapses, in vitro, within 1 min.

(2) During a fixed 5-min incubation with γ-32P-ATP, the optimal incubation time with 10 nM T3 is maximally stimulatory protein phosphorylation was between 40-120 sec. The 96 and 79 kDa proteins responded more to the T3-induced phosphorylation as compared to the 114 kDa protein.

(3) In conclusion, brain physiological concentrations of T3 rapidly enhanced protein phosphorylation in in vitro synaptosomes, a nuclease-free subcellular fraction from young adult rat brain cerebral cortex, in vitro. This finding suggests a novel nongenomic signal transduction pathway for T3 in adult mammalian brain.

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