# NONGENOMIC ACTIONS OF THYROXINE ON CYTOSKELETON PHOSPHORYLATION IN CEREBRAL CORTEX OF YOUNG RATS ARE MEDIATED BY INTRACELLULAR CALCIUM LEVELS AND KINASE PATHWAYS

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The developmental effects of thyroid hormones (TH) in mammalian brain are mainly mediated by nuclear receptors regulating gene expression. However, there are increasing evidences of nongenomic mechanisms of these hormones associated with kinase- and calcium-activated signaling pathways. In this context, the aim of the present work was to investigate the signaling pathways involved in the mechanism of action of TH on cytoskeletal phosphorylation in cerebral cortex of 15-day-old rats. Tissue slices were incubated with $^{32}P$ orthophosphate in the presence or absence of $T_3$, $T_4$ or specific inhibitors of phospholipase C and protein kinases for 30 min. The intermediate filament (IF) enriched cytoskeletal fraction was obtained and the $^{32}P$ incorporated into IF proteins was measured. Results showed that $T_4$ increased the IF phosphorylation without altering the total immunocontent of these proteins. Otherwise, neither $T_3$ nor neurotransmitters (GABA, ATP, glutamate or epinephrine) acted on the cytoskeletal-associated phosphorylating system. We also demonstrated that the mechanisms underlying the $T_4$ effect on the cytoskeleton involve membrane initiated actions through $G_i$ protein-coupled receptor, as evidenced by using pertussis toxin or H-89 ($G_i$ protein and PKA inhibitors, respectively) and reinforced by the inhibition of cAMP levels. Moreover, we showed the participation of PLC, PKC, MAPK, PKCaMII and intracellular $Ca^{2+}$ levels mediating the effects of $T_4$ on the cytoskeleton by using specific inhibitors and a calcium quelator (U73122, stearoilcarnitine chloride, PD98059, KN-93 and BAPTA-AM, respectively). Stimulation of $^{45}Ca^{2+}$ uptake by $T_4$ was also demonstrated. These findings suggest that $T_4$ has important nongenomic actions modulating the cytoskeleton of neural cells during development.

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