

Thyroxine signal transduction in liver cells involves phospholipase C and phospholipase D activation. Genomic independent action of thyroid hormone

ABSTRACT

The present data have indicated the DAG signaling in thyroid hormone-stimulated liver cells. L-thyroxine activates a dual phospholipase pathway in a sequential and synchronized manner: phospholipase C initiates the DAG formation, and PKC mediates the integration of phospholipase D into the signaling response during the sustained phase of agonist stimulation.

INTRODUCTION

Background Thyroid hormone exerts a broad range of effects on development, growth and metabolism. The actions of thyroid hormone are primarily the result of their interaction with nuclear receptors that bind to regulatory regions of genes (thyroid hormone - response elements) and modify their expression. Nuclear mechanisms of thyroid hormone action have been extensively described [reviewed in], but an increasing number of nongenomic effects of the hormone at the cellular level have been recognized in the past 10 years [reviewed in]. Nongenomic actions of thyroid hormone are by definition independent on nuclear receptors for the hormone and have been described at the plasma membrane, various organelles, the cytoskeleton, and in cytoplasm. The actions include alterations in transport of Ca^{+2} , Na^{+} and glucose; changes in activities of several kinases, including protein kinase C (PKC), cAMP -dependent protein kinase and mitogen - activated protein kinase. Iodothyronines also can regulate nongenomically through a PKC activation of neutral lipids, phospholipids and phosphatidylinositol 4,5-bisphosphate (PtdIns (4,5)P_2) synthesis in rat hepatocytes. It has been determined that in HeLa cells potentiation by thyroxine (T4) interferon -gamma - induced antiviral state requires PKC and phospholipase C (PLC) activities. Direct evidence of the nongenomic PKC activation by thyroid hormones has been found in rabbit erythrocytes. The regulation of PKC is critical to the mechanism by which thyroid hormones rapidly induce phosphorylation and nuclear translocation of mitogen-activated protein kinase and subsequently potentiate both the antiviral and immunomodulatory actions of $\text{IFN}\gamma$ in cultured cells. It is widely demonstrated on various cell types that interaction of Ca^{+2} - mobilizing hormones and transmitters with the cell surface receptors leads to the phospholipid breakdown under PLC or -D action and accumulation of inositol phosphates and diacylglycerol (DAG). The regulatory molecules generation is accompanied by intracellular free calcium concentration increase and, as a result, by PKC activation. An addition of the physiological doses of thyroid hormones to the cell suspension rapidly increases the intracellular calcium concentration in rat hepatocytes and single rat heart cell. On the other hand, there is no information about accumulation of other PKC modulator - DAG in the cells on T4 administration. However, such genomic independent effect on the different types of cells has been determined for steroid hormones whose mechanism of action on target cells is known to be similar to that of the thyroid hormones. In the present study, we have examined the nongenomic effect of thyroid hormones on DAG formation and PKC activation in liver cells. It was determined that L-T4 rapidly induces the biphasic DAG accumulation in liver slices and isolated hepatocytes. The data obtained provide evidence that L-T4 activates PLC and -D in sequential manner that leads to increasing DAG formation during sustained

agonist stimulation. The L-T4-induced PLD -PA phosphohydrolase (PAP) pathway of DAG generation in rat hepatocytes is highly specific and PKC - dependent.

CONCLUSION

Conclusions The investigations made indicate that in liver cells L-T4 rapidly stimulates the hydrolysis of polyphosphoinositides by PLC with the resultant production of the second messengers inositol triphosphate as well as DAG and PKC activation. The major new finding of this study was that in hepatocytes L-T4 stimulated PC cleavage by PLD. As in the other cells, operated by Ca²⁺-mobilizing receptors, PLD contributes to DAG formation in L-T4-stimulated hepatocytes. DAG formed by PA breakdown could further activate PKC in hormone-treated cells. Inhibitor of PLC-dependent phosphoinositide hydrolysis, neomycin sulfate, completely abolishes the first phase of DAG production and reduces the PLD-dependent DAG response to L-T4, indicating that PLD is activated during the PLC-dependent signaling in liver cells. These data indicate that L-T4 activates a dual phospholipase pathway in a sequential and synchronized manner. PLC initiates the increase in Ins(1,4,5)P₃ and DAG formation and PKC mediates integration of PLD into the signaling response during the sustained phase of agonist stimulation. The effect of L-T4 on PKC, PLD activation and DAG accumulation is highly specific and too rapid (from seconds to a few minutes) to be compatible with mRNA and protein synthesis. These results provide the first evidence concerning L-T4 nongenomic stimulation of phospholipid hydrolysis by phospholipases and DAG accumulation in liver slices and isolated hepatocytes.