Thyroid disruptor 1,1,1-trichloro-2,2-bis(p-chlorophenyl) ethane (DDT) prevents internalization of TSH receptor

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Abstract The thyroid-stimulating hormone (TSH) receptor (TSHr) was made specifically fluorescent by insertion of a tetracysteine motif (TSHr-FIAsH) into the C-terminal end and transiently transfected into COS-7 and HeLa cells. The observation that TSH administration caused the intracellular level of cAMP to increase in both TSHr-FIAsH-transfected cell types indicated that the FIAsH binding motif did not alter normal TSHr functioning. When transfected into HeLa cells and stimulated with TSH, the TSHr-FIAsH receptor exhibited a pronounced perinuclear labelling pattern, whereas labelling remained on the cell surface following pre-incubation with 1,1,1-trichloro-2,2-bis(p-chlorophenyl) ethane (DDT). Chinese hamster ovary (CHO)-TSHr cells probed with anti-TSHr antibodies were fluorescent mainly in the proximity of the plasma membrane, with fluorescence being primarily restricted to a juxta-nuclear position when exposed to 10 mU/ml TSH for 1 or 5 min. However, in the presence of DDT, the anti-TSHr fluorescence maintained a peripheral location along the cell plasma membrane, even if CHO-TSHr cells were stimulated with TSH for 1 and 5 min. To verify that DDT acted specifically

on the TSHr, CHO cells transfected with the A_{2a} receptor were used as controls. Following a 1-min stimulation with 5'-(N-ethyl-carboxamido)-adenosine, A_{2a} receptors were gradually internalized regardless of the presence of DDT in the culture medium. Finally, immunoelectron microscopy of CHO-TSHr cells showed that a 1-min exposure to TSH sufficed to displace anti-TSHr antibodies tagged with 10-nm gold particles into coated pits and vesicles but that their superficial location was retained along the plasma membrane in the presence of DDT.

Keywords Thyroid-stimulating hormone receptor · Endocytosis · 1,1,1-Trichloro-2,2-bis(p-chlorophenyl) ethane · A_{2a} receptor · Chinese hamster ovary cells

Introduction

Much of what is presently known about the mechanisms controlling cell proliferation and differentiation stems from the knowledge that cells can express a number of surface receptors capable of interacting with the external milieu. Depending on the nature of the ligand-receptor interaction, cells may respond specifically to a variety of signals carried by many of the information-coding molecules present in the extracellular environment (Maggio et al. 2007). About 80% of the hormones, neurotransmitters and growth factors present in the bloodstream of eukaryotic organisms controls a plethora of metabolic responses by interacting specifically with G-protein-coupled receptors (GPCRs) (Bockaert and Pin 1999). Within the large family of the GPCRs, the glycoprotein hormone receptors specific for luteinizing hormone, follicle-stimulating hormone and thyroid-stimulating hormone (TSH) share the common characteristics of having a long ectodomain comprised of numerous leucine

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