5 Summary

Conditions of pathological thyroid growth are extremely common, and currently they are one of the most frequently encountered problems in endocrinological practice. While TSH and its mediator cAMP are well-established regulators of normal thyroid growth, the last decade has provided evidence that pathological growth may instead involve disturbances in tyrosine kinase and PKC activities. In this study, the activation of CRE-binding protein family of transcription factors upon exposure to established thyroid growth factors was examined. CREB/M phosphorylation was found to occur in a time- and dosage-dependent manner, not only upon induction of cAMP signalling with forskolin and TSH, but also upon stimulation with TPA, EGF or insulin, the latter requiring a minimal concentration of 1 µg/mL, indicating the employment of IGF receptor types. Semiquantitative RT-PCR of ICER and transient transfection with a CRE-reporter construct were employed to investigate transcriptional activation upon CREB/M- phosphorylation. Surprisingly, there was a striking difference in the kinetics of TSH/forskolin induction of CRE-mediated gene induction (5-fold increase, maximal at 8 hours), and EGF/TPA (2.5-fold, maximal at 24 hours). Application of specific kinase inhibitors revealed the involvement of PKA and PKC in CREB/M-KID phosphorylation by both types of mediators, whereas neither p70S6K nor Camk II were found to be required. Interestingly, the response towards TSH was biphasic, with the first phosphorylation peak seen at 10 min and coinciding with maximal sensitivity to the PKA inhibitor H89, and the second peak seen at 30 minutes with simultaneously maximal sensitivity to PKC inhibition by GFX. In contrast, during EGF- mediated CREB/M-phosphorylation PKC and PKA appeared to act within a single pathway. Comparing CRE-binding factor activation in thyroid primary cells with that seen in several thyroid carcinoma cell lines, an increasing reactivity towards EGF was found to accompany a concomitant loss to TSH/insulin-responsiveness and differentiation. Taken together, the results of this study thus support the idea of TSH/insulin and PKA being the main mediators in normal and benign thyroid growth, whereas EGF and PKC are the predominant triggers in malignant transformation.