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Development of a High-Throughput Screening Assay for Serotonin Receptors

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AIM

The present study describes the development of a high throughput screening system using stably transfected insect cell lines overexpressing the human serotonin receptor type 4a (h5-HT4a), which is positively coupled to adenylyl cyclase, and a reporter gene (CAT, Chloramphenicol Acetyl Transferase, or GFP, Green Fluorescent Protein) under the control of a regulatory element which provides transcriptional activation specificity and high expression levels. This high throughput screening system aims at the identification of "lead compounds" which will be useful for the development of new drugs acting on the CNS.

METHODS

An increase of the intracellular levels of cAMP results in the activation of protein kinase PKA, phosphorylation and subsequent activation of transcription factor CREB (CRE-Binding Protein) followed by binding of CREB to the CRE (cAMP Response Element) regulatory sequence and activation of target genes. In order to determine the levels of activation of this signaling pathway after h5-HT4a stimulation, reporter plasmids were constructed, in which the CAT reporter gene was placed under transcriptional control of CRE and the silkworm basal actin promoter. The h5-HT4a cDNA was placed under transcriptional control of an enhanced promoter to induce protein expression, and the levels of the expressed receptor were determined using a binding assay for membranes of transiently transfected cells. The efficiency of h5-HT4a coupling to endogenous Gs proteins and its ability to stimulate adenylyl cyclase activity after receptor activation by serotonin in transiently transfected insect cells was also tested.

RESULTS

Expression of the serotonin receptor in these cells resulted in an increase of CAT activity both in the presence or absence of serotonin, which is indicative of constitutive receptor activity.

CONCLUSIONS

The above results suggest that the use of specific expression vectors can facilitate heterologous functional expression of mammalian membrane receptors in insect cell lines. Development of such a high throughput screening system for serotonergic analogs in insect cell lines will aid in the discovery of new bioactive molecules from plant extracts which act as inverse agonists or the human serotonin receptor.