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cAMP Down-Regulates α_{2C}-Adrenergic Receptor at the Transcriptional Level in HepG2 Cells

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AIM

The α_2-adrenergic receptors (α_2-ARs) mediate the effects of epinephrine and nor-epinephrine into the cell. They are members of the G-protein coupled receptors superfamily and their stimulation leads to inhibition of adenylate cyclase and decrease of cAMP levels. The human α_2-ARs exist as three pharmacological subtypes, α_{2A}, α_{2B} and α_{2C}. Studies of the α_{2C}-AR have been hampered by the lack of a cellular system natively expressing this subtype. The HepG2 hepatocarcinoma cell-line was recently found to natively express this subtype and studies in this cell-line have shown that long-term agonist treatment results in down-regulation of α_{2C}-AR in these cells, due to increased receptor protein degradation. Based on this finding, the aim of this study was to investigate the heterologous regulation of the α_{2C}-adrenergic receptor (α_{2C}-AR) by cAMP in HepG2 cells.

MATERIALS AND METHODS

HepG2 cells were cultured in DMEM under standard conditions or subjected to treatment with different modulators (forskolin, 8-br-cAMP etc.). Levels of α_{2C}-AR were determined by ligand binding assays with [^{3}H]MK912 (α_2-antagonist) on membranes prepared from cells submitted to different treatments. Steady state levels of α_{2C}-AR mRNA were determined by RNase protection assays. Measurements of luciferase activity, after cell transfection with reporter gene constructs, were carried out to determine the *cis* acting elements of the α_{2C}-AR promoter necessary to confer sensitivity to the cAMP effect. To characterize the transacting factors that mediate the effect of cAMP, gel retardation experiments were carried out, as described.

RESULTS

Exposure to forskolin or analogs of cAMP resulted in a significant reduction of α_{2C}-AR number in a dose-dependent manner. This reduction of receptor density is not due to an increased rate of receptor degradation, but is the result of a decrease in the steady state amounts of α_{2C} -AR mRNA, which was found to be primarily due to decreased rate of α_{2C}-AR gene transcription. Based on these results, we have started to characterize the *cis* and *trans* acting elements involved in the repression of the α_{2C} -AR gene transcription by cAMP. Transfection experiments with reporter gene constructs containing different fragments of the 5′-flanking region of the α_{2C}-AR gene showed the existence of a *cis* acting ele-

ment, within a 240 bp fragment of the promoter, which confers sensitivity to the cAMP effect. Although this region does not contain any consensus sequence, it includes a CRE-like element (TGCCATCA). Initial results from gel-retardation experiments indicate that there is a rather specific time-dependent and forskolin-activated transcription factor(s) binding to this region and that the CRE-like element is not necessary for this binding.

CONCLUSIONS

cAMP down-regulates the α_{2C}–adrenoceptor in HepG2 cells by decreasing the rate of transcription of the α_{2C}-AR gene. It appears that cAMP exerts this effect by inducing binding of nuclear factors to the α_{2C}-AR gene promoter in a time-dependent manner. These findings bring novel insight into the mechanisms employed by cAMP in the down-regulation of the α_{2C}–AR.