Upstream open reading frames regulate nicotinic acetylcholine receptor subunits associated with smoking and smoking-related disorders

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Introduction

Nicotine addiction poses a major health problem worldwide and is known to considerably increase the risk for diseases such as cancer and cardiovascular pathologies. Nicotine both modulates nicotinic acetylcholine receptor (nAChR) subunit expression in various, mostly still unknown ways and acts as a receptor ligand. The genes coding for nAChRs are therefore suspected to play a key role concerning smoking behaviour and related disorders. Especially post-transcriptionally regulatory mechanisms are considered to be involved in the modulation of nAChR subunit expression by nicotine such as internal ribosomal entry sites, microRNA-binding sites and upstream open reading frames (uORFs) located within the untranslated regions (UTR).

For CHRNA4 isoform 2 and CHRN3 uORFs no significant results were obtained. As to CHRNA4 isoform 1 and CHRN5, qPCR did not show a significant difference in mRNA quantity when comparing intact versus switched-off uORFs (Fig. 2 B and Fig. 3 B). Therefore, we could rule out for both genes that the luciferase assay results were due to a transcriptional effect.

Material and Methods

We performed a systematic search for functionally relevant uORFs in the 5′UTR of the nAChR genes CHRNA3, CHRNA4 isoform 1 and 2, CHRN5, CHRN7 and CHRN3 (Fig. 1). The 5′UTR of the nAChR subunits a4 isoform 1 and 2, a5 and 83 were cloned into the vector pGL4.10+TK. Constructs with intact start codons were compared to constructs lacking the start codon (ATG mutated to TTG). HEK293 cells were co-transfected with the above-mentioned pGL4.10+TK constructs and the control plasmid pGL4.74. We measured the firefly and renilla luciferase activities by Dual-Glow luciferase assay. The mRNA quantity of firefly and renilla luciferases were assessed by qPCR. The ratio of firefly luciferase and renilla luciferase activity was calculated and normalized to pGL4.10+TK. The various 5′UTR constructs are expressed as fold change to pGL4.10+TK (Fig. 2 and 3).

Discussion

The data presented here strongly suggest that uORFs within the 5′UTR of CHRNA4 isoform 1 and CHRN5 are important regulators of protein translation. Interestingly the uORF found to be functional in CHRNA4 isoform 1 is also present in CHRNA4 isoform 2 but does not seem to reduce gene expression in the latter sequence context. A possible reason could be the presence of additional, so far unknown regulatory motifs that only exist in the long 5′UTR of isoform 2. To our knowledge this presents the first example of an uORF with isoform-specific functional relevance.

Results

Reporter gene assays revealed that the uORFs of CHRNA4 isoform 1 and CHRN5 are able to significantly downregulate the luciferase protein expression and are thus functional (Fig. 2 A and Fig. 3 A).

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