Intron retention as an alternative splice variant of the rat urocortin 1 gene

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Abstract

Urocortin 1, highly conserved metazoan gene of the corticotropin-releasing hormone family, is a simple gene structured in two exons and the corresponding intron. The urocortin 1 prepropeptide is entirely coded in the second exon. Preliminary non-isotopic *in situ* hybridization experiments with an oligonucleotide complementary to an intron sequence of the urocortin 1 gene showed a significant cytoplasmic-like staining, suggesting the occurrence of an intron-retained urocortin 1 transcript. This observation prompted us to study whether the urocortin 1 gene presents alternative splicing by intron retention event. Confocal fluorescent *in situ* hybridization for urocortin 1 RNA and the use of the specific DNA dye TOPRO-3 allowed us to show significant expression of the intron-retained urocortin 1 transcript that did not colocalize with TOPRO-3 staining indicating a cytoplasmic localization for the intron-retained urocortin 1 transcript. The natural occurrence of a polyadenylated intron-retained urocortin 1 RNA was further documented by reverse transcriptase polymerase chain reaction (PCR), primed with oligo(dT), of total RNA extracted from three brain regions, a midbrain region containing the Edinger-Westphal nucleus, cerebellum and prefrontal cortex. In the three brain regions studied, it was possible to amplify both intron-less as well as intron-retained urocortin 1 transcripts. The use of PCR primers that simultaneously amplify both urocortin 1 transcripts allowed us to show that the expression of both urocortin 1 transcripts differs among the brain regions analyzed, suggesting a tissue specific regulation of this alternative splicing. In silico analysis of the five known mammalian urocortin 1 genomic sequences showed high conservation of the urocortin 1 intron sequence. Further studies should investigate the regulation of this intron retention event and its consequence for the functionality of the urocortin 1 gene.