Review paper

Biological indications of a novel "short" μ opiate receptor in domestic chicken

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Abstract

Previous work from our laboratory has established that cellular signaling processes of endogenous morphine are mediated by cognate G protein coupled receptor (GPCR) proteins, designated μ_3 and μ_4 opiate receptors. μ_3 and μ_4 opiate receptors are structurally unique "short" 6 transmembrane helical (TMH) domain GPCRs that are selectively responsive to endogenous morphine, not to families of endogenous opioid peptides, and are uniquely coupled to activation of constitutive nitric oxide synthase (cNOS). Based on high resolution predictive measures, it appears likely that domestic poultry express a μ opiate receptor mRNA encoding potentially two novel GPCRs with similar biochemical characteristics as described for μ_3 and μ_4 opiate receptors as well as traditional μ_1 opioid receptors. The biological indications of these novel μ opiate receptors are discussed within the context of this short review.

Key words: endogenous morphine, chicken μ opiate receptor, G protein coupled receptor, transmembrane helical domain.

Introduction

Previous work from our laboratory has focused on the elucidation of biochemical, cellular, and molecular mechanisms underlying the regulatory roles of endogenously expressed, chemically authentic, morphine in animal cells and organ systems [1-9]. As a critical corollary, we have established that cellular signaling processes of endogenous morphine are mediated by cognate G protein coupled receptor (GPCR) proteins, designated μ_3 and μ_4 opiate receptors. μ_3 and μ_4 opiate receptors are structurally tailored to be selectively activated by morphine and morphine-related opiate alkaloids and not by related families of endogenous opioid peptides [10, 11] and are functionally coupled to activation of constitutive nitric oxide (NO) production and release [3, 6, 8, 9, 12-14].

The unique structural features of μ_3 and μ_4 opiate receptors are determined post-transcriptionally via selective splicing of the primary transcript of the μ_1 opioid receptor (MOR) gene [1, 15-17]. Mature μ_3 and μ_4 opiate receptor-encoding mRNAs translate into receptor proteins lacking an amino acid sequence of approximately 90 amino acids that constitute the extracellular N-terminal and transmembrane helical (TMH)1 domains and part of the first intracellular loop of the μ_1 receptor, but retain the empirically defined ligand binding pocket distributed across conserved TMH2, TMH3, and TMH7 domains of the μ_1 sequence. In effect, μ_3 and μ_4

opiate receptors are "short" 6TMH domain GPCRs that are selectively responsive to endogenous morphine.

Predictive measures indicate a novel "short" μ opiate receptor in domestic chicken

Our compelling demonstration of novel "short" 6 TMH domain μ opiate receptors stimulated an exhaustive search of existing databases to determine whether additional 6TMH domain u opiate receptors were expressed in various animal species [17]. The National Center for Biotechnology Information (NCBI) database yielded a predicted chicken μ opioid receptor mRNA sequence that provided putative evidence supporting the existence of a novel "short" μ opiate receptor in the domestic chicken. Interestingly, the 5' end of predicted mRNA sequence was observed to contain three potential ATG start codons (Figure 1). Consensus sequence analysis of probable translation initiation site (TIS) was performed as according to Kozak sequence guidelines, as previously described [18-23]. Accordingly, predicted nucleotide sequences utilizing the first and the third initiation codons as likely TIS candidates were translated into two probable protein sequences of 327 and 300 amino acids, respectively, using the Translation Web Tool provided by EXPASY.

Conformational analysis of the smaller 300 amino acid μ opiate receptor protein species, performed by computer program TMHMM, indicated a 6TMH domain GPCR with an identical membrane topology to native μ_3 and μ_4 opiate receptors (Figure 2A) [16, 17]. Interestingly, computational analysis of the larger 327 amino acid μ opiate receptor protein species indicated a novel 7TMH domain GPCR lacking a typical extracellular domain containing consensus N-linked glycosylation sites (Figure 2B). In both cases, predictive measures indicate that the domestic chicken expresses one or two novel "short" GPCRs with similar biochemical characteristics as previously described for μ_3 and μ_4 opiate receptors as well as traditional MOR's.

Finally, BLAST (Basic Local Alignment Search Tool)-mediated comparative amino acid sequence analysis yielded an N-terminal sequence homology of 96% for an alignment containing amino acid residues 1-290 of the predicted 300 amino acid chicken μ opiate receptor in comparison to amino acid residues 1-290 of the 292 amino acid μ_4 opiate receptor [16, 17]. BLAST analysis also yielded a C-terminal sequence homology of 96% for an alignment containing amino acid residues 1-300 of the predicted 300 amino acid chicken μ opiate receptor in comparison to amino acid residues 101 to 400 of the 400 amino acid μ_1 opiate receptor. Thus, the 300 amino acid 6 TMH domain chicken μ opiate receptor may be operationally defined as an N-terminally truncated "short" homolog of the μ_1 opioid receptor (Figure 3). Due to the novel 6 TMH domain configuration and sequence identity to μ_3 and μ_4 opiate receptors at its N-terminus, we predict that the 300 amino acid chicken μ opiate receptor functions as a "hybrid" signaling GPCR with selective preference for morphine and related morphinan alkaloids with the exclusion of endogenous opioid peptides [10, 11]. Similar criteria relating to ligand selectivity will most certainly apply to the predicted novel "short" 7TMH domain 327 amino acid chicken μ opiate receptor due to the genetic deletion of the glycosylated extracellular N-terminal domain.

Biological indications of a novel "short" μ opiate receptor in domestic chicken

Based on guidelines established by Kozak *et al*. [22, 23] it appears that mature chicken μ opiate receptor-encoding mRNA contains two probable TISs with the potential for translation of two distinct 300 and 327 amino acid μ opiate receptor proteins. Prior literature indicates that many mRNAs are capable of producing functionally distinct proteins using different in frame start codons within the same mature fully spliced mRNA [20, 24]. Furthermore, the effects of upstream start codons can vary with cell type during differentiation [25-28]. Accordingly, mature chicken μ opiate receptor-encoding mRNA may be similarly translated into one or two functional receptor proteins that are sorted or expressed according to tissue or cell type. Morphine and other chemically related opiate alkaloids represent classical and reliable analgesic principles for management of severe pain associated with disease [5, 8, 9, 12, 14, 29-43]. Paradoxical morphine-mediated hyperalgesia in the presence of typical morphine-mediated respiratory depression has been observed in the domestic chicken [44-46]. It is a reasonable, therefore, to speculate that these markedly different physiological responses to administered morphine may be due differential expression of the 300 vs.

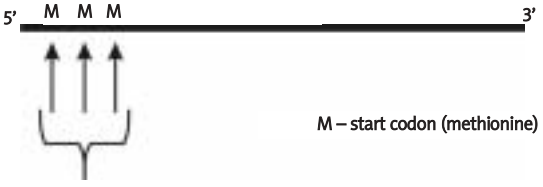

Figure 1. Full length untranslated mRNA species from novel chicken opioid receptor has the potential for three different start sites, the first will produce a protein species equivalent to 37 kDa, and the last will produce a protein species equivalent to 34 kDa

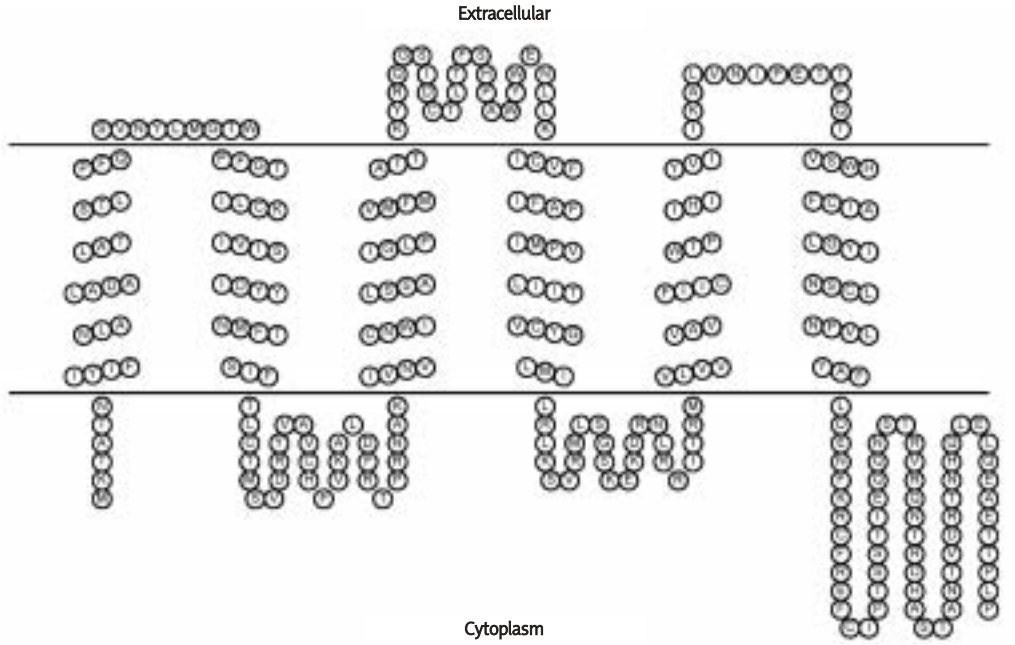

Figure 2A. Graphic representation of the predicted 300 amino acid receptor species based on the previously shown TMHMM predictions and determinations. Image was provided by the Sequence Analysis and Consulting Service using TOPO2 [52] software funded by the University of California, San Francisco, USA

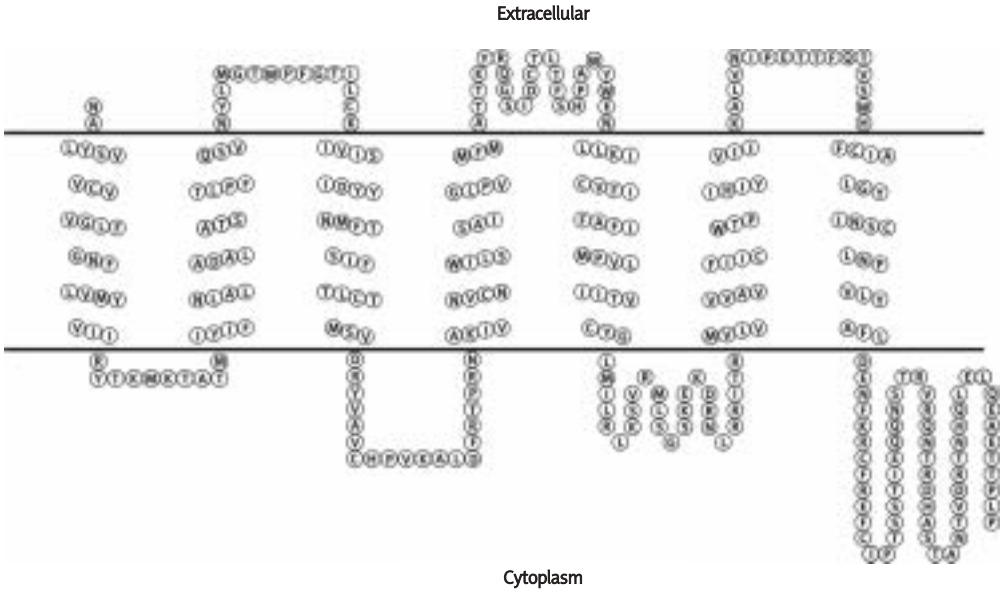

Figure 2B. Graphic representation of the predicted 327 amino acid receptor species based on TMHMM determinations. Image was provided by the Sequence Analysis and Consulting Service using TOPO2 [52] software funded by the University of California, San Francisco, USA

the 327 amino acid μ opiate receptor in spinal cord and brain stem loci.

Conclusions

The presence of this biologically unique, functional "short" membrane bound receptor protein in the chicken not only reinforces the primacy of said receptor but in doing that it also gives us view into a window of learning and understanding the history of evolution. The chicken is evolutionarily placed to bridge the gap between mammals and non-amniote vertebrates, and is therefore the best studied representative of all avian species, thus providing a valuable resource for understanding comparative genomics [47]. Interestingly, inspection of the chicken MOR gene

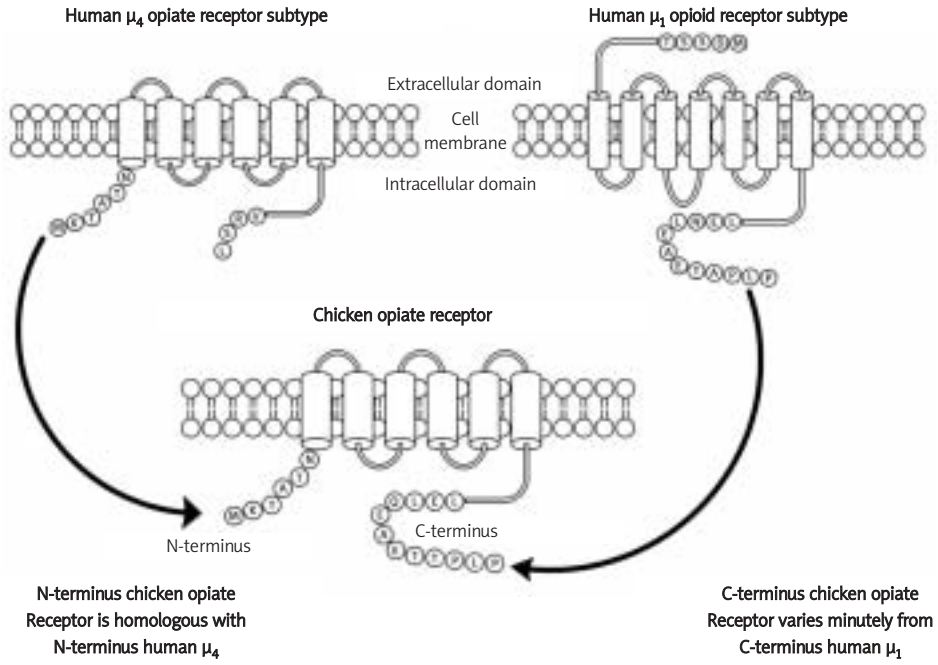

Figure 3. Schematic representation of the predicted amino acid sequence of the 300 amino acid 6 TMH domain chicken μ opiate receptor in comparison to the 6 TMH domain μ_4 opiate receptor and the traditional 7 TMH domain μ_1 opioid receptor. The 300 amino acid 6 TMH domain chicken μ opiate receptor may be operationally defined as an N-terminally truncated "short" homolog of the μ_1 opioid receptor. The novel 6 TMH domain configuration and sequence identity to μ_3 and μ_4 opiate receptors at its N-terminus suggest that the chicken μ opiate receptor functions as a "hybrid" signaling GPCR with selective preference for morphine and related morphinan alkaloids with the exclusion of endogenous opioid peptides. Illustration was designed using ChemBioOffice 2008, Cambridgesoft, Cambridge Massachusetts, USA

indicated a very streamlined nucleotide sequence. The link between genome size and metabolic rate was first made in 1970 by Henryk Szarski [48-50]. Birds have the smallest genome when compared to other vertebrates including humans [51]. Avian species require a high metabolic rate to carry out basic physiological functions. The metabolic advantage of a smaller, relatively streamlined genome within all cells allows cells not only to be smaller but operationally expands cellular surface area to volume ratios. Smaller genomes do not necessarily mean fewer genes, but rather a more succinct use of space on the chromosomes. In effect, smaller cells are more energy efficient that larger ones, resulting in an increased metabolic advantage. These complementary data may also contribute to understanding the physiological role of nucleated red blood cells in birds [16, 17]. It is possible that avian species due to their relatively streamlined genome and smaller size do not require expulsion of the nucleus from the red blood cells before its entry into the blood stream. In effect, the high surface to volume ratio of nucleated red cells in avian species facilitates markedly efficient gas exchange with surrounding tissues. Future studies to elucidate the physiological role of novel μ opiate receptors in these same metabolic processes are highly necessitated by our initial findings.

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