An amphibian model for investigation of opioid analgesia and pain-processing (3-Dec-2002)

C. W. Stevens

Oklahoma State University, Center for Health Sciences, College of Osteopathic Medicine, Tulsa, OK, USA.

Our research focuses on investigating the mechanisms of opioid analgesia and vertebrate pain-processing using an amphibian model. For scientific, ethical, and economical reasons, we spent the last 12 years developing and establishing an alternative or adjunct model for opioid and pain research using the Northern grass frog, *Rana pipiens*. Our initial studies focused on characterizing the behavioral response of frogs to the application of dilute acetic acid to the animal’s hindlimb and the attenuation of this response by the administration of opioids and other analgesic agents. A series of behavioral studies measuring the antinociceptive effect of opioids administered by systemic and central routes established the finding that the relative analgesic potency of opioids in amphibians was highly correlated to the relative potency of the same opioids noted in mammalian studies and to that observed in humans in clinical studies. These early studies established that the amphibian model, using the acetic acid test as an algesiometric assay, is a robust and predictable assay for opioid action and could serve as adjunct model.

Other behavioral studies examined the time course and magnitude of morphine analgesic tolerance, the opioid mechanisms of stress-induced analgesia, seasonal changes in opioid sensitivity, and the sensory modalities altered by opioid administration. Additionally, the relative potency of alpha-adrenergic agents was assessed after both central and peripheral administration. Finally, the sensitivity of the acetic acid test to modulation following other potential non-opioid analgesics such as benzodiazepines, cholinergics, histaminergics, and barbiturates was determined and reported on.

More recently, in an effort to build a hierarchal approach to investigating opioid action in amphibians, we carried out studies to characterize the opioid binding proteins (opioid receptors) expressed in amphibian brain and spinal cord using radioligand binding techniques in tissue homogenates. These results demonstrated that although at the behavioral level opioid analgesia in amphibians and mammals appear very similar, the characteristics of opioid binding sites are slightly different in the two vertebrate species. The first data with the general opioid antagonist, naloxone, suggested that there was a single binding site that mediated the analgesic effects of the three types of opioids, *mu*, *kappa*, and *delta*. This led to an intriguing hypothesis termed the unireceptor hypothesis in which it was thought that a single opioid receptor was expressed in earlier-evolved vertebrates. Further studies using selective *mu*, *kappa* or *delta* opioid agonists showed that amphibian brain contains distinct binding sites for each type of opioid, in apparent contradiction to the data obtained with the general opioid antagonist radioligand, naloxone.

The final phase of our hierarchical approach to the investigation of opioid analgesia in amphibians was to launch a major project to clone and sequence putative opioid receptor proteins from *Rana pipiens*. This latest effort has yielded three fully sequenced opioid receptor proteins with close homology to *mu*, *kappa*, and *delta* opioid receptors previously cloned in mammals and humans. The addition of non-mammalian opioid receptor sequences using an amphibian tissue source to existing databases of opioid receptor sequences allows us now to examine the conservation of particular domains of the opioid receptor protein in an attempt to assign regions of the primary structure that confer type selectivity to opioid receptors. In this regard, the use of the amphibian model provides for the first time a comparative approach to the bioinformatics of opioid receptor sequences and a new approach to address questions of the detailed mechanisms of opioid analgesia in all vertebrates including humans.
Reference List for the Amphibian Model

Peer-Reviewed Publications:

Book chapters and reviews


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