AN APPROACH TO TARGETED THERAPY: SYNTHESIS AND BIOLOGICAL ACTIVITY OF HYDROPHOBIC AND HYDROPHILIC ENKEPHALIN ANALOGUES

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INTRODUCTION

The mechanisms whereby opioid peptides (naturally occurring peptides with opiate-like biological properties) activate processes on their target receptors are still at an early stage of exploration. The aim of receptor research is to purify and isolate receptor glycoproteins or glycolipids in order to further implement the knowledge of the physicochemical aspects involved in the interaction of opioids with their receptor.

Opioid peptides like other neuroactive peptides, are derived from large molecular weight proteins and are synthesized with ribosomal participation. To date, it is now clear that there are at least three different families of endogenous opioid peptides: the enkephalins, the endorphins and the dynorphins, that may act as neurotransmitters or neurohormones. It is also well established that these various opioid peptides are derived from three biosynthetic precursors: the Proenkephalin-A, that contains 6 copies of [Met]-enkephalin and one copy of [Leu]-enkephalin; the Pro-opiomelanocortin, that contains the sequence of the \( \beta \) -endorphin; and the Prodynorphin that includes the sequences of the dynorphins. The primary structures of these precursors have been deduced by the use of recombinant DNA techniques. It is possible to isolate and purify the pro-hormone mRNA from hormone rich tissues. DNAs complementary to the mRNAs encoding these precursors have been cloned and the determination of their nucleotide sequences has lead to the elucidation of the amino acid sequences of the precursor proteins.

The physiological action of these peptides is mediated by Opioid Receptors. Such receptors consist of both a recognition site, to which the peptide binds, and a nervous device that translates the binding into biochemical events that lead to a biological response. Three different subtypes of opioid receptors: (\( \mu \), \( \delta \) and \( \kappa \)) have been characterized so far. Each of them are supposed to mediate different physiological effects. Thus, \( \mu \) receptors appear to be involved in analgesia and more precisely in heat mediated nociception. They are supposed to mediate the respiratory depressant effects of opiates as well as the inhibition of...
intestinal motility. The development of physical dependence seems to be mediated by the \( \mu \) binding sites; \( \delta \)-receptors seem to be involved in respiratory depression and circulatory shock. It is still not clear whether \( \delta \)-receptors are involved in analgesia; \( \kappa \)-receptors, on the contrary, seem to mediate analgesia and, particularly, pressure nociception. They are also involved in diuresis and feeding behaviour.

The concept of multiple Opioid Receptor subtypes offers a new strategy for the development of targeted therapy if specific "in vivo" effects can be undoubtedly associated with the occupancy of specific receptors.

Design of opioids with analgesic efficacies dissociated from respiratory depression or the development of physical dependence is obviously of the utmost importance. In this regard, there is evidence that the administration of a \( \delta \)-selective antagonist, reversed hypotension associated with endotoxin shock, without altering the levels of analgesia induced by morphine, which is supposed to interact with the \( \mu \)-subtype (Holaday et al. 1982).

To go further in this objective of "targeted therapy" it is of great importance to obtain information on the chemical requirements of each binding site. To date the best way to obtain information concerning the chemical structure of the opioid receptors is to conduct investigations based on physicochemical principles that would help in the isolation of the binding sites. Such studies should be complementary to those dealing with the in situ properties within the membrane.

As far as the isolation of the opioid receptors is concerned it is worth remembering that many efforts have been made in this direction by either the two major approaches to the isolation of surface receptors. In the first a labeled ligand is bound to the receptor, and the ligand binding site complex is solubilized and isolated; in the other, the binding sites are solubilized and isolated in the absence of opioids. However, nearly all attempts have failed and the studies on the isolation of the opioid binding sites are still at an early stage.

One of the most interesting studies to date in this respect is that by Gioannini et al. 1984, who found that opioid binding sites solubilized from bovine striatum were specifically retained by affinity chromatography on a wheat germ agglutinin agarose column. Although wheat germ agglutinin not only recognizes N-acetyl glucosamine residues and also sialoproteins, further studies with specific lectins for sialic acid did not retain any of the solubilized binding sites. These results suggested that the extracted material might be a glycoprotein containing N-acetyl glucosamine residues.

On the other hand, in our laboratory, the present authors could demonstrate, by an indirect approach, using monolayer techniques and studying the profiles of the different isotherms, that gangliosides, a group of sialic acid containing glycosphingolipids, were involved in the chemical structure of the opioid receptors. By monolayer techniques one can easily examine the interactions between neuronal membrane lipids and opioid agonists and antagonists. Lipid monolayers may be arranged at an air-water interface using a Langmuir balance (Albretch, 1983). The interactions between the monolayer components and solutes included in the aqueous subphase may be examined through measurement of the lateral compressibility of the monolayer (Phillips et al., 1968). Mixed monolayers, of various lipids prepared from the stock solution and spread over a range of surface concentrations, yield isotherms which are dependent upon the molar fraction of each lipid. When examined at low