Overview

The Molecular Structure of Opiate Receptors*

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Examples are given which demonstrate that the κ opiate receptor can be separated from μ and δ subtypes by their physical parameters. When the subunit composition of the subtypes are compared, no definite differences are encountered. The data from the literature are also contradictory. This may in part be explained by the fact that the different receptors appear to contain a structurally common high affinity binding site. A possible hypothesis would be that the subtypes differ from each other by the number of subunits.

KEY WORDS: Opiate receptors; subtype specificity; subunit structure.

L'opium agrandit ce qui n'a pas de bornes,
Allonge l'illimité,
Approfondit le temps, creuse la volupté,
Et, de plaisirs noirs et mornes,
Remplit l'âme au delà de sa capacité.
(Charles Baudelaire)

Molecular pharmacology of receptors started in the seventies when receptor binding assays were developed to measure the binding of labeled ligands to the receptor. Beside equilibrium and non equilibrium measurements to determine binding constants the other goal of molecular "receptorology" was to establish the primary, secondary and tertiary structure of receptors. The first procedural step in such studies consists of solubilizing the receptor out of membranes and its purification (generally by affinity chromatography), followed by partial determination of the amino acid sequence of the pure protein and/or nucleotide sequence of the receptor gene. Finally, in order to convincingly demonstrate that the receptor structure determined is correct and functional, it is necessary to reincorporate the isolated protein into membranes, to demonstrate that binding of the ligand is accompanied by the anticipated ionic permeability changes or by other biological parameters of receptor activation. Therefore, as the proof of the pudding is in eating, reconstitution of the purified receptor into the membrane is necessary in order to establish the receptor function of the binding protein.

The nicotinic acetylcholine receptor isolated from Torpedo was the first neurotransmitter receptor which was purified and sequenced (1, 2) followed by the hormone receptors of insulin (3) and EGF (4); many others are now in progress.

One of the receptors whose structure has not yet been elucidated is that of the opioid binding protein. The difficulty is due in part to the many ligands which can bind to the receptor. These range from alkaloids of plant origin, synthetic compounds (like benzomorphan) to many peptides (enkephalins, endorphins and dynorphins) present endogenously in the nervous system (5-7).

The Subtypes of the Opiate Receptor. In correspondence with the multiplicity of the ligands, the
receptors also exhibit a similar variety (μ, δ, κ, σ, ε) even though the specificity of the natural endogenous ligands of some of them is not yet firmly established (μ, σ) (8 and 8a). Besides exhibiting specific pharmacological and physiological effects when activated—as f.e. analgesia (μ and κ opioid receptors) sedative action (κ receptors) anti-endotoxic shock (δ receptors)—the distribution of the various subtypes of opioid receptors in the nervous system also seems to differ in part. For example, guinea pig cerebellum contains only the κ subtype (9) while the NG 108–15 hybrid cell line is a representative of δ opioid receptors (10). Another means of distinguishing between the opiate receptors is by the presence or absence of high and low affinity binding sites. For example μ and δ receptors have a common high affinity binding site which is called μ1 and is supposed to be responsible for the analgesic effect of their ligands (11).

From the pharmacological and biochemical experiments the following questions were raised: Do the subtypes represent distinct proteins or is a single opiate receptor subtype defined by different combinations of identically structured sites? Might the application of different ligands induce dissimilar conformational changes in the same protein? Finally, are the subtypes composed of different monomers in the oligomer or polymer of opioid receptor?

There are several experimental facts which confirm the first hypothesis, namely that the subtypes are in fact distinct molecules. Localization experiments have clearly indicated that κ and δ receptor subtypes occur in certain areas where other subtypes are absent (9, 10). The molecular weight of the digitonin solubilized complex has been determined at 600,000–875,000 for μ and δ but the M. wt. for the κ opioid receptor subtype in guinea pig brain is only 400,000 (9). Experiments performed on Sepharose 6B chromatography columns (Figure 1) and sucrose density ultracentrifugation resulted in complete physical separation of the κ subunit in the solubilized frog brain. The estimated molecular weight of the receptor digitonin complex of this subunit was 300,000 (12). Tritiated ethylketocyclazocine (EKC) binding was not displaced in the κ fraction by DAGO or DALA and EKC serves as the only effective ligand among many substances tested (Figure 2).

Chow and Zukin (13) separated also the κ opioid receptor subtype from μ receptors in rat brain preparations solubilized with CHAPS on Sepharose CL-6B columns. They detected two types of κ populations only one of which coincided with the μ receptor while the other one represented a different protein. The δ subtype was not demonstrated in their preparation. The Stokes radius of their separated subtype was 50 Å, whereas the Stokes radius of the other fraction, solubilized with digitonin from frog brain was 64 Å (12).

Using irradiation inactivation techniques in rat brain slices (14) and membranes (15) a M.wt. of