Unit IV: Pharmacodynamics:

Receptors and receptors subtypes

The delineation of multiple types and subtypes of receptors for signal molecules has played an important role in the development of a number of targeted and more selective drugs. Even at an early stage of evolution of receptor pharmacology, it was observed that actions of acetylcholine could be grouped into '**muscarinic**' and '**nicotinic**' depending upon whether they were mimicked by the then known alkaloids muscarine or nicotine. Accordingly, they were said to be mediated by two types of cholinergic receptors, *viz.* muscarinic or nicotinic (N); a concept strengthened by the finding that muscarinic actions were blocked by atropine, while nicotinic actions were blocked by curare. In a landmark study, Ahlquist (1948) divided adrenergic receptors into 'α' and 'β' on the basis of two distinct rank order of potencies of adrenergic agonists. These receptors have now been further subdivided (M1, M2 ….M5), (NM, NN) (α1, α2) (β1, β2, β3). Multiple subtypes of receptors for practically all transmitters, autacoids, hormones, etc. are now known and have paved the way for introduction of numerous clinically superior drugs. In many cases, receptor classification has provided sound explanation for differences observed in the actions of closely related drugs.

The following criteria have been utilized in classifying receptors:

1) Pharmacological Criteria

Classification is based on relative potencies of selective agonists and antagonists. This is the classical and oldest approach with direct clinical bearing; was used in delineating M and N cholinergic, α and β adrenergic, H1 and H2 histaminergic receptors, etc.

2) Tissue Distribution

The relative organ/tissue distribution is the basis for designating the subtype, e.g. the cardiac β adrenergic receptors as β1, while bronchial as β2. This division was confirmed by selective agonists and antagonists as well as by molecular cloning.

3) Ligand Binding

Measurement of specific binding of high affinity radio-labelled ligand to cellular fragments (usually membranes) *in vitro*, and its displacement by various selective agonists/antagonists is used to delineate receptor subtypes. Multiple 5HT receptors were distinguished by this approach. Autoradiography has helped in mapping distribution of receptor subtypes in the brain and other organs.

4) Transducer Pathway

Receptor subtypes may be distinguished by the mechanism through which their activation is linked to the response, e.g. M cholinergic receptor acts through G-proteins, while N cholinergic receptor gates influx of Na^+ ions; α adrenergic receptor acts *via* IP3-DAG pathway and by decreasing cAMP, while β adrenergic receptor increases cAMP; $GABA_A$ receptor is a ligand gated Cl^- channel, while $GABA_B$ receptor increases K^+ conductance through a G-protein.

5) Molecular cloning

The receptor protein is cloned and its detailed amino acid sequence as well as three dimentional structure is worked out. Subtypes are designated on the basis of sequence homology. This approach has in the recent years resulted in a flood of receptor subtypes and several isoforms (which do not differ in ligand selectivity) of each subtype. The functional significance of many of

these subtypes/ isoforms is dubious. Even receptors without known ligands (orphan receptors) have been described.

Application of so many approaches has thrown up several detailed, confusing and often conflicting classifications of receptors. However, a consensus receptor classification is now decided on a continuing basis by an expert group of the International Union of Pharmacological Sciences (IUPHAR).

6) Silent Receptors

These are sites which bind specific drugs but no pharmacological response is elicited. They are better called *drug acceptors* or *sites of loss*, e.g. plasma proteins which have binding sites for many drugs. To avoid confusion, the term receptor should be restricted to those regulatory binding sites which are capable of generating a response.

The receptor subtype is also defined by the pharmacological characteristics of the site and is based on the availability of selective agonists and antagonists for the subtypes.

For example, **Beta adrenergic receptors (ADRBs)** are **transmembrane G-protein-coupled receptors** that bind adrenalin or noradrenaline in sympathetic nervous system. There are three types of ADRBs: ADRB1, ADRB2 and ADRB3. The amino acid similarity of the transmembrane regions is high among the subtypes of a given receptor type (often in the range of 65% for G-protein-coupled receptors), but it is lower among receptor types in the same family (often in the range of 40%). ADRB3 has been least studied to date and the role of ADRB3 in cardiovascular disease is not known. ADRB1 are the predominant type expressed in the hearth. ADRB2 are abundantly expressed in bronchial smooth cells and activation of them results in bronchodilatation.

The **ligand-gated ion channel receptors** comprise four or five subunits, each of which may have several isoforms. Thus, the potential number of receptor subtypes is quite large. For many of these receptors, it is not yet clear which of the many possible combinations are actually presented in vivo. Because in some cases pharmacological specificity appears to reside in a single subunit, receptor subtypes can be defined by the various isoforms of that particular protein.

Estrogen receptors (ERs) are **intracellular nuclear receptors** that belong to steroid hormone receptor family. There are two types of ER; ER alpha (ER 1) and ER beta (ER 2) encoded by two different genes ESR1 and ESR2, respectively. ESR1 and ESR2 are expressed mostly bones, breasts, ovaries, cardiovascular system and central-neural system, but ESR2 mRNA was also found in kidney, lung, colon and testis tissues. ERs function as transcription factors activated by a ligand. Ligands bind to ERs are endogenous estrogen hormones (estradiol) or estrogens administered at hormone replacement therapy (HRT). A new group of drugs, "selective estrogen receptor modulators" (SERMs) act as ER-agonist in a specific tissue (like raloxifen in bone) but as an ER-antagonist in other tissue (like tamoxifen in breast). Irrespective of ligand, ligand binding to ER results in activation and translocation of ERs to nucleus, where the complexes bind to specific DNA sequences (estrogen responsive element). In Association with other coactivators and represors alters the expression of target genes. For example binding of ER1-ligand complex to fos/jun complex facilitate the binding of fos/jun heterodimer to AP-1 site and activation of many genes including IGF-1. On the contrary, ESRs inhibit binding of NF-kB to IL-6 promoter.