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Significance of pharmacological study of neurotransmitter receptors in brain research

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Studies of the various aspects of receptor structure and their modulation by pharmacological agents represent a major area of current thrust in neurosciences at the cellular and subcellular level as well as at the behavioural level. The pharmacological criteria for receptor characterization have been used extensively not only to classify the receptors but also to understand the physiological roles of various neurotransmitters and help in the development of new drugs. The information obtained using procedures of molecular biology is complementary and a proper characterization of a receptor needs both types of information.

The thrust area for neurotransmitter receptor research should include basic studies to obtain receptor in a pure form, techniques to solubilize them, mapping of neurotransmitter receptors in healthy and diseased brain and their interactions with drugs. Ligands need to be discovered for orphan receptors, and the suitability of using peripheral receptors in lymphocytes, platelets, etc., as well as invertebrate receptors as indicators of CNS receptors should receive top priority in research.

THE term receptor has been used by pharmacologists and physiologists for over a century, but the concept, the definition and the functions have undergone major changes during the last decade. There has been a tremendous growth in information about drug–receptor interactions and about receptor *per se*, and in 1987 the International Union of Pharmacological Sciences appointed a Committee to rationalize the nomenclature of receptors. The Committee has, in principle, accepted a broad-based operational definition of receptor. Briefly stated, a receptor must recognize a distinct chemical entity and translate this information into a form that the cell can read and alter its overt state by initiation of a biochemical process, by alteration in an ongoing biochemical process or by modulation of the action of an indigenous effector. This definition ascribes the function of both recognition and transduction to a receptor.

Historical development of the receptor concept

Waldeyer in 1891 coined the term neurone[1] and Langley in 1909 further developing the concept of drug–receptor interaction, stated that drugs could form a dissociable complex with the receptor and that receptive substances (receptors) on cells had a clearly defined physiological function[2]. Around the same time, Hill[3] analysed the action of nicotine and curare on frog skeletal muscle to provide evidence that drug action could be antagonized also by interaction at the receptor level.

Most of the early studies, even though employing pharmacological agents, were qualitative. Clark[4,5] pioneered the concept of dose–response relationship and initiated a quantitative study of drug–receptor interaction. Schild[6] further extended the quantitation to drug antagonism at receptors and used the negative log of molecular concentration of an antagonist (pA$_x$), which would reduce the effect of multiple dose of an agonist to that of a single dose in the absence of the antagonist[7].

Importance of receptor study in neurobiology

Studies of the various aspects of receptor structure and function and their modulation by pharmacological agents constitute a major and vital part of the current thrust area in neuroscience research. This may be broadly divided into the following main areas.

Molecular neurobiology

The following research problems are covered under this subject:

1. Assay of molecules associated with transmitter synthesis, storage, release and response, particularly for the rapidly expanding number of neuropeptides.
2. Amino acid sequence of neurotransmitter receptors to distinguish ion channel receptors from signal transductors, to identify the sites of action of endogenous ligands and to provide natural template for rational drug design. This is beyond the purview of the present discussion.
3. Regulation of growth factors, initiating or preventing repair and plasticity responses.

Cellular neurobiology

Cellular studies are vital for the understanding of neuronal interactions. At the interneuronal level, they will be involved in information processing involving transduction mechanisms. They are also important for analysing neuronal–glial interactions.

Major attention has been paid to the vertebrate nervous system. It is now being realized that brain neural organization in the non-vertebrates is much simpler and the receptor studies are easier to conduct. Attention is also being paid to neurotransmitter receptors in pathogenic helminths like ascaris, filaria, etc.

Behavioural research

Receptor study has contributed significantly to the understanding of the integration of neurones, glia and vasculature into an ensemble for behavioural tasks. They are valuable for understanding the regulation of autonomic activities and immune functions by the central nervous system (CNS) and in elucidating the site and mechanism of drug action.

Pharmacological criteria for receptor characterization

The use of agonists and antagonists plays an important role in elucidating the receptor type and its function, the major emphasis being on the ability of antagonists to prevent or reverse agonist-induced or physiological response. A major advantage in using antagonists is the utilization of *in vitro* assay procedure of the type developed by Gaddum[8] or Schild[6], or radioligand-binding assay[9], but measurements must be made under equilibrium conditions and the regression should be linear with unit slope over a considerable concentration range[10]. There can be several complicating factors, some of which are equally so in the use of agonists. The three major complicating factors are:

(i) Receptors sufficiently precoupled to G-proteins and antagonists may interfere with receptor–coupling-protein interaction[11].

(ii) The antagonist may bind to only one of the several binding domains for an agonist, each of which may be classified as a unique receptor.

(iii) The agonist affinity may be modified by receptor environment due to flexibility of the receptor, specially if transduction occurs.

The relative potency of several agonists in the same tissue can be used as a receptor fingerprint, but the picture may change if different tissues are involved and several agonists are used. The main complicating factors in such a situation are:

(i) *Intrinsic activity.* Ariens[12] postulated that dose response depends on affinity as well as on intrinsic activity. According to this concept, a full agonist will have an intrinsic activity of 1, a complete antagonist will be devoid of intrinsic activity and a partial agonist will be in between.

(ii) *Spare receptors.* Nickerson[13] showed that with lower concentrations of irreversible antagonists the dose–response curve shifted to the right but the maxima remained unchanged, whereas with very high concentrations the maxima might not be achieved. This was explained on the basis of spare receptors.

(iii) *Transduction.* In several cases agonist binding to the receptor may be non-cooperative[14], and the effect is transmitted to events regulating a response by a transducer molecule[15], which may act either by a biochemical mechanism, e.g. involving a second messenger, or by a conformational change, like opening of an ion channel.

(iv) Stereochemical considerations have got to be constantly taken into account while using drugs for receptor analysis. The enantiomeric pairs are not only good probes for details of a cell surface, they also may help in discriminating between receptor subtypes.

Some case studies

During the last two decades, we have made extensive use of pharmacological agents to characterize CNS receptors. A few case studies are being presented here to bring out the advantages of such an approach.

The earliest study[16] on characterization of adrenoceptors involved in facilitation of the flexor reflex using specific agonists, a- and b-adrenoceptor antagonists and partial agonists established the presence of a-adrenoceptors. A battery of a- and b-agonists and antagonists was also employed to characterize similarly the adrenoceptors involved in thermoregulation in rabbit, found to be a-adrenoceptors[17]. In pigeon a-adrenoceptor stimulation produced hypothermia. Interestingly, dopamine effect was shown[18] to be mediated by two types of receptors, one blocked by haloperidol and the other by ergometrine. To demonstrate the physiological role of these neurotransmitters, the effect was studied in *Mastomys natalensis* at different ambient temperatures[19]. The involvement of dopamine receptor could also be demonstrated in hypothermic effect of amphetamine in the *Mastomys*[20]. The involvement of adrenoceptors in central cardiovascular control has also been analysed. Employing techniques like microinjections, topical application, effect on reflex and direct exactability of central vasomotor locii, central lesions and selective adrenoceptor antagonists or toxins like 6-hydroxydopamine, an excitatory role of a-adrenoceptors has been demonstrated at various sites[21–25].

Studies were also initiated to utilize peripheral receptors for predicting the status of CNS receptors. Thus, platelet dopamine receptors were studied in 40 cases of Parkinson's disease. Two subpopulations of patients were detected, one with a significantly reduced number of receptors and the other with a much higher number. The patient of the second subgroup showed a good response to L-Dopa therapy and the binding returned to normal. Similarly, a poor response in the other group was associated with little change in dopamine binding[26].

Lack of space precludes a discussion of other similar studies undertaken by us to characterize the receptors involved in stereotyped behaviour[27–29] and in regulation of blood brain barrier[30], and to analyse the central hypotensive effect of clonidine[23, 24, 31].

Use of newer technologies for receptor characterization

Several powerful technologies have become available during the last 10–15 years which can be utilized effectively for characterization of the CNS receptors. A detailed discussion is not possible but a listing of major useful technologies is given below:

1. PET. *Position emission tomography* can be conveniently used to study the distribution of suitably labelled biologically active natural ligands and thereby help in quantitation and elucidation of biochemical characteristics of neurotransmitter receptors in the brain, including in the human brain.

Similar studies with labelled drugs will lead to a better understanding of the differences in receptor density and/or affinity between the responders and the non-responders and of more basic information about concentration versus receptor alterations in disease states.

2. *Magnetic imaging.* Magnetic resonance imaging (MRI) gives better resolution than PET for mapping of receptors binding to specific drugs, e.g. benzodiazepines. Neuromagnetic imaging (NMR) helps in study of changes in neural signals due to brain activities and their modification by drugs acting on specific receptors. The SQUIDs have been particularly useful. NMR and MRI can be judiciously combined for dual imaging.

3. *Computer modelling.* This has been utilized extensively and for diverse purposes. It has helped in identification of pharmacophones and three-dimensional receptor structure and in analysing drug–receptor interactions. It has also been valuable in designing of rigid analogues and biosters of agonists and antagonists for a better understanding of the receptor conformation and function.

Thrust areas for receptor research

In 1988 the National Advisory Mental Health Council of NIMH submitted a report[32] to the US Congress listing 50 important questions to be answered during the next decade (the decade of the brain). It is significant that 12 of these are directly or indirectly related to studies on neurotransmitter receptors. The thrust areas for receptor research are:

1. *Basic studies.* (i) Major effort is required to obtain receptors in a pure form and use techniques to solubilize them. This will help in identification and characterization of the binding sites and in better understanding of the drug and neurotransmitter interactions with the receptor. (ii) A detailed receptor mapping for various neurotransmitters and neuromodulators in normal as well as diseased brain is important. (iii) The receptors involved in modulation of brain activity by neuropeptides, hormones and microglial secretion need to be identified and characterized, and their interaction with the neurotransmitters/drugs studied.

2. *Physiological and pathological roles*, specially in cotransmission, consolidation of memory and mental illnesses.

3. *Peripheral receptors* e.g. in platelets, lymphocytes, etc., as indicators of central receptors as well as receptors in invertebrates.

4. *In vitro receptor interaction* for development of new drugs, using a battery of neurotransmitter receptor assays.

5. *Orphan receptors.* Identification of suitable natural ligands.

Concluding remarks

The foregoing review indicates clearly that a judicious use of pharmacological agents can not only help rationally characterize the receptors but also contribute materially to elucidate the physiological role of the various neurotransmitters and receptor subtypes. Such studies are valuable in analysing drug action and in generating data for rational synthesis of more effective and selective drugs. A number of new powerful tools will complement these pharmacological studies to generate better and more expeditious information.

The system of pharmacological characterization will continue to be important since it establishes the unique pharmacological profile based on response to specific agonists and antagonists. It also demonstrates the presence of an endogenous agonist and possible physiological effects of receptor stimulation using a variety of models, from conscious animals to *in vitro* preparations, as has been demonstrated clearly in the foregoing section. At the same time, in the last decade a vast amount of information has been obtained from the alternative method of molecular biology for the study of receptor structure by determining the amino acid sequence and cloning. This has resulted in identification of a very large number of receptor types and subtypes, many without even an identified ligand and, therefore, termed as *orphan receptors*. The physiological role of such receptors also remains obscure. A complete characterization of receptor will need both types of information, since they are complementary to each other.

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