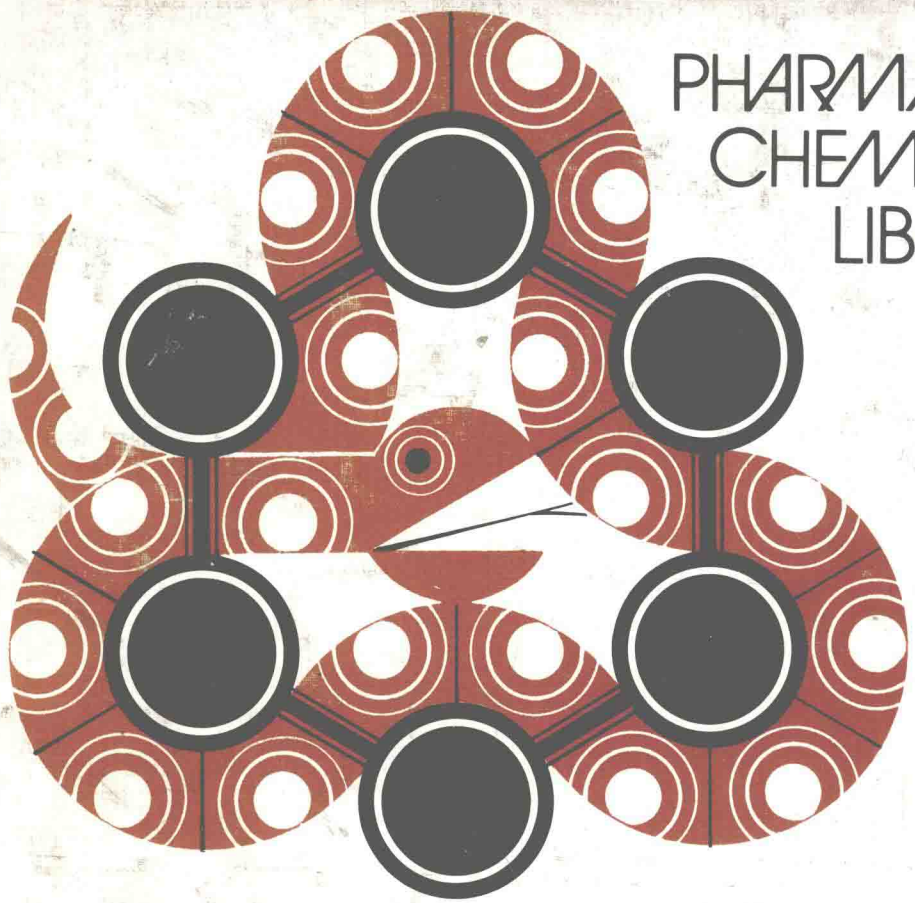

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INNOVATIVE APPROACHES IN DRUG RESEARCH

EDITED BY

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INNOVATIVE APPROACHES IN DRUG RESEARCH

Proceedings of the third Noordwijkerhout Symposium on Medicinal Chemistry, held in The Netherlands, September 3–6, 1985

Edited by

A.F. HARMS

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Preface

The series Noordwijkerhout Symposia on Medicinal Chemistry is organized by the Medicinal Chemistry Division of the Royal Netherlands Chemical Society (KNCV). The third symposium "Innovative Approaches in Drug Research" has been sponsored by the International Committee on Medicinal Chemistry (ICMC), the International Union of Pure and Applied Chemistry (IUPAC), the International Union of Pharmacology (IUPHAR), the European Federation for Medicinal Chemistry (EFMC), the Fédération Internationale Pharmaceutique (FIP), the Royal Netherlands Chemical Society (KNCV), the Royal Netherlands Association for the Advancement of Pharmacy (KNMP) and the Dutch Society for Pharmacology.

The major aim of the series is to intensify the dialogue between the two groups of scientists active in the field of medicinal chemistry: the chemists including the (Q)SAR-ists, and those who work mainly on biological aspects.

The third symposium was held during the period September 3-6, 1985 in Congres Center Leeuwenhorst in Noordwijkerhout, the Netherlands; about 300 participants from over 20 countries attended the symposium.

Under the heading "Innovative Approaches in Drug Research" the organizers had selected the following subjects: Receptors and preliminary screening in drug development; Drugs and macromolecules, structures and modelling; New developments in chemotherapy; Natural products and medicinal chemistry; Topics of current interest. The programme included 20 main lectures, 7 short communications and 62 poster presentations.

During the several lectures it became clear that the time has come to leave some of the traditional routes in drug research and especially in medicinal chemistry. Modern developments such as DNA-recombinant techniques (e.g. for receptor research) and computergraphics are making themselves felt and are growing in importance.

As satellites of the symposium two separate sessions took place on "the role of stereochemistry in the development of new selective drugs" and "S.A.R. and design of anti-tumour agents".

In the opening lecture by Sir Arnold Burgen on "the road to rational drug design" much emphasis was laid on new ways of lead finding. From this lecture it could be concluded that productive research for the development of new drugs can only be attained by way of an interdisciplinary approach. The meeting showed that many medicinal chemists have already understood this message.

These proceedings provide the complete texts of all main lectures given at the symposium and its satellite sessions.

H. Timmerman
Chairman organizing committee

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A.F. Harms (Editor), *Innovative Approaches in Drug Research*

THE ROAD TO RATIONAL DRUG DESIGN

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ABSTRACT

Classical methods of drug discovery are essentially empirical. Increasingly new paradigms are needed for drug development based on peptides and proteins and the structure of drug receptors. The problems of moving to this level of complexity are discussed.

Rational drug usage and development may be regarded as having started 180 years ago with the isolation of the first plant alkaloid, morphine. There followed a period in which many alkaloids were isolated and in consequence a glimmering of the relationship between chemical structure and drug action developed. A parallel strand of development stems from the synthetic chemical industry in which many new substances were tested for biological activity and active substances found. The development from there was from the lead compound through the synthesis of modified compounds and the generation of optimised structures. This was essentially a game of chance, in which serendipity and experience played their part, and in recent years we have attempted to improve the odds by methods from QSAR to model building and molecular mechanics.

When such a new drug action is discovered, the process then begins of trying to define the intimate mechanism of action, which takes us to the discovery of the molecular targets of the drug action, whether receptors or enzymes or some non-specific targets. At this stage, the problem can often be redefined in terms of the endogenous agents acting at this site, which may take us to a new series of agents based on the endogenous compound acting as a lead.

Sufficient exploration may lead to the discovery of complementary drugs such as agonist-antagonist pairs. Since we are largely considering relatively simple compounds, in general their specificity for a single receptor may be rather low, and through

amplification of apparent side actions entirely new series of drug actions may be developed. A famous example of this kind of drug discovery is the sulphonamide series from sulphanilamide, through chlorothiazide to the sulphonylureas.

While the kind of familiar logic detailed here has led to the development of a high proportion of the drugs in current use and represents a great achievement, there is no doubt that it is a wasteful method, with both failures and disasters on its path and is intellectually not very satisfying.

An alternative road of development stems from the isolation of constituents of the animal body, a parallel to the natural products of the vegetable kingdom but in which the agents are directly operative in the animal body. In some sense this has an inverted logic from the method discussed previously.

We take some simple or partly purified material from animal sources and find what it does, and then purify out the active material and discover its structure. This is essentially the methodology of endocrinology which has developed separately but in parallel with pharmacology. The hormones so discovered offer the opportunities for the chemist though often as we will see pose different and more complex problems. The discovery of neuro-transmitters represents a parallel discovery of what have been called local hormones. We are now seeing the discovery of many kinds of substance which in their different ways fit into a hormonal category which I will generalise to be the production of a substance by one cell that influences the action of other cells. I am thinking of the cellular control substances, such as erythropoietin, nerve growth factor and colony stimulating factors. Boundaries between the three classes of substance I have mentioned are really very artificial - just consider for instance the action of catecholamines at a synaptic junction or released into the circulation from the adrenal medulla!

What these substances are relevant to, is the totally pervasive and fundamental property of all living systems - self regulation. No complex organism can function without an elaborate system of control mechanisms that ensure that the various functions of the organism are kept in step with each other and the external demands on the organism. It is obvious that such regulation in a mammal will operate at a series of levels controlling gross

functions such as blood flow and extracellular electrolyte concentrations as well as cell size, division and differentiation and the more subtle regulation of perception and cognition. Nowhere is the evidence of regulation more evident than in development - superintending the development of a complex organism from a single cell. This alone indicates to us that with all the wonderful discoveries of recent years we understand but a tithe of the directive influences that must be involved in cell differentiation and intercellular relations. Nevertheless, all these processes are regulated by chemical mediators acting on the appropriate receptors and hence are the potential sources of new drug development - what a rich future lies ahead if we can find the ways to exploit it! Let me give an example from cancer research. It was found some years ago that a proportion of breast cancers were oestrogen dependent and that if antioestrogens such as tamoxiphen were administered the tumours ceased to grow - whether or not the tumours were sensitive depended on the presence of oestrogen receptors. The evidence grows that most tumour cells are dependent on cell growth factors that have the normal function of controlling the division and commitment of cell populations; for instance, colony stimulating factors are necessary for the development of some marrow stem cells into mature cell populations and these same factors can cause leukaemic cells in culture to change into mature cells. The discovery of more about such systems is likely to lead to more promising methods of dealing with cancer than the crude interference with nucleic acid metabolism that is the basis of much of our current armamentarium.

Let us assume that we have already many promising targets to study and many more are on the way, what should be our strategy in converting these into therapeutic agents?

We are faced with the difficulty that many are moderate sized proteins. Nowadays that is no great obstacle to preparation although the effort that was required to prepare interferons (an example really of such agents) in quantity should not be underestimated. I will assume that the first strategy is to reduce the size of the proteins in the hope that moderate sized peptide fragments retain activity as has fortunately been the case with gastrin and some other polypeptide hormones, although this does not necessarily make them into generally useful therapeutic

agents, largely because of unfavourable pharmacokinetics.

The general solution to the problem is to find some way of translating the surface properties of the biologically active part of the polypeptide surface into an equivalent non-peptide; in a nutshell, this is the problem of discovering morphine when you only know about enkephalin!

There is no general solution available at present although a number of strategies can be considered. The first is the one that has proved so successful in the converting enzyme inhibitor, replacement of amino acids by stages with other structures that conserve the geometry and physical properties. As is well known, in the successful agents captopril and enalapril all but the terminal proline has been replaced, and there are newer compounds in which even the proline has been replaced.

To carry such a development through with reasonable efficiency we need to know the conformation of the peptide and the portion of its surface involved in the interaction. Calculation of the conformation of a small peptide in vacuo by energy minimisation methods is achievable, the conformation in aqueous media rather more uncertain. Measurement by NMR in aqueous and non-aqueous solvents can give information about well-populated conformations and may be a simpler approach. Much information about some of the peptide hormones such as vasopressin has been obtained by this method. The parts of the surface involved need to be found by chemical modification either by removing potentially interactive groups or introducing sterically interfering groups. A nice example of this has recently been reported for repressor-DNA interactions.

However, it is likely that in many cases the biological activity will not survive extensive truncation and we will be forced to model the surface of moderate sized proteins. Even here some of the tools for such a study are available. A good deal of information as to which amino acids are on the surface can come from hydrophobicity data - most of the hydrophobic residues will be interior, and also from high resolution NMR data particularly using paramagnetic reagents to define the residues in contact with the solvent. Further information can be obtained by monoclonal antibodies directed against relatively short peptide sequences from the protein.

These may also give useful information about which faces of the protein are involved in its biological activity. This is achieved by examining which monoclonals (or their Fab fragments) interfere with the activity. Site-directed mutagenesis will also give information of which amino acids in the active surface are important. The monoclonals against oligopeptides in the structure make us consider a paradox. If no oligopeptide from the protein shows biological activity why should we expect it to tell us anything about the active site?

The likely answer is that the monoclonal occludes the active site which includes other residues than those in the immunoactive peptide that are essential for binding to the receptor.

Why have I not mentioned the obvious solution - x-ray crystallography? Certainly it is the ideal method, but it remains a difficult method even for rather small proteins with many barriers to success. What about predicting protein conformation? This has very great promise. At present the main method available is based on the behaviour of peptide sequences derived from a library of crystal structures of proteins (essentially the rules first described by Chou and Fasman) and these can be taken together with all the other pieces of information mentioned abobe together with other physical information such as circular dichroism. The result obtained would be imperfect and some way short of what we could hope to obtain from x-ray crystallography but it might well provide enough clues about the major interactive sites and their disposition in space for some speculative chemical synthesis. What is badly needed is a crystallographic technique which defines the envelope of a macromolecule without the necessity to solve for all the atomic coordinates.

This problem of obtaining three-dimensional structures of proteins is the major stumbling block to development in drug design. We see at the present time the sequence of receptors being described at an increasing rate thanks to the greatly improved methods for isolating receptors present in very small amounts and for determining their sequence through hybridising the c-DNA corresponding to relatively short peptide sequences with a gene library and subsequent clonal replication. We are

thereby presented with peptide sequences of receptors. These are fascinating achievements but what help will they be to drug design unless we can solve the problems of three-dimensional structure? The problems are likely to be more difficult than with the protein regulators if only because of the larger size of the peptide; however, it is fortunate that we now have ample evidence that purified receptors are active and can have much the same ligand binding properties in solution as in membranes. We have good examples, such as the insulin receptor, where antibodies not only will block the receptor, but will actually activate the receptor. The first paper has already appeared on site-directed mutagenesis. However, I believe that the earliest benefits from acquiring sequences are likely to be the assignment of receptors into families including the definition of how sub-types of receptors differ. If one can obtain high resolution x-ray diffraction data on one member of a receptor family it seems probable that taken with the other evidence referred to earlier it may be possible to construct three-dimensional models that give useful information about the binding site.

I have so far treated receptors in a restricted sense, but if one includes enzymes there are now a considerable number for which high resolution diffraction data are available and in many cases including inhibitor or pseudo-substrate complexes. These provide the opportunity for analysing the binding area into a contour of varying charge and hydrophobicity on which to model inhibitors, with the ability to calculate the energetic contributions of individual atomic interactions.

Let us take a simple example, the binding of benzene sulphonamides to carbonic anhydrase depends on forming an inner sphere complex with the zinc at the enzyme active centre supported by hydrophobic interactions with one flank of the active site cleft. The hydrophobic interactions both provide extra binding energy and also orientate the sulphonamide group. It should be possible to predict from the contours of the hydrophobic surface the optimal substitutions to maximise binding. More interestingly, other substituents might be introduced to take advantage of binding to the other rather hydrophilic face of the cleft. I am not aware that this has been done. More

complex problems exist with other enzymes such as dihydrofolate reductase but require exploring to develop our feel for this kind of problem; indeed the study of systems where empirical drug development has led to excellent compounds, as in these two cases, is especially valuable.

These explorations should be taken further to see if they cannot be used for designing totally novel drugs to fit some other part of the site. Study of enzyme reactions using crystal structures has reinforced the idea of transitional state complexes being strongly bound states and has led to the introduction of pseudo-transitional groups in drug synthesis. My impression is that medicinal chemists are still not using enzyme structures sufficiently. No doubt because the enzyme structures they would most like to study are as yet unavailable. I would argue that so much can be gained from studying unfashionable enzymes in terms of liberalising our thoughts from proceeding along well-worn and rather threadbare tracks that they should be strongly supported.

Finally, let me put in a word for the use of isolated receptor studies in drug development. They offer a much lower level of fundamental information than the methods I have been discussing but they may have several virtues; they are rapid and cheap and enable the operation of a very broad screen, they concentrate effort on a particular action, and through a battery of receptor assays can provide valuable information on selectivity and even some light on mechanisms.

You will say that after being in the stratosphere I have come down to earth with a bang and recognised that for many years yet drug discovery will remain essentially empirical and dependent on chance. But, one day!

A.F. Harms (Editor), *Innovative Approaches in Drug Research*
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RECEPTORS: A TOOL IN DRUG DEVELOPMENT

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INTRODUCTION

Life, in a way, can be considered as a highly organised dynamic complex of chemical processes. Organisation requires regulation and this in its turn communication, conveyance of information at various levels of organisation: intracellular, intercellular and between the organism and its milieu externe. To a large extent, the communication is based on messenger molecules which in their chemical, structural properties are coded for recognition by and activation of particular molecular sites of action, the receptors. Examples are neurotransmitters, involved in direct cell to cell communication, mediators which convey information mainly by diffusion between groups of cells, covering relatively small distances, and hormones released by particular, glandular, tissues and acting on distant organs, being transported predominantly by convection, e.g. the blood stream.

The capacity of the messenger molecules to recognize their specific receptors has its origin in a chemical complementarity, often visualized as a lock and key relation but actually being much more dynamic. It is a mutual moulding, an "embracement", of messenger and receptor in which various intermolecular forces are involved. The messenger molecule has an affinity for and undergoes binding to its specific receptors. As a result of the interaction the receptors are activated, their conformation being changed in such a way that a sequence of chemical and physico-chemical processes are initiated which finally lead to the effect observed. Besides an affinity to the receptors the messenger, an agonist, has an intrinsic activity on the receptors, its efficacy. The action of many therapeutics is based on interference with messenger-receptor interactions. The drug may act as an agonist, a messenger-"mimetic"; it then has an affinity and an intrinsic activity, or it may act as a competitive antagonist of the messenger, as a receptor blocker; then it has an affinity to the receptors but lacks the intrinsic activity (1-5).

Therapeutics, drugs, have a messenger function too. They are exogenous messengers by means of which the physician communicates with the organism, interfering, hopefully in a corrective way, with particular messenger-receptor interactions. In some cases, like that of morphine, the receptors were detected before the corresponding endogenous messenger was known. After the receptors had been recognized, displacement experiments led to the discovery of the endogenous messengers, the endorphines (6,7). Similarly the interaction of cardiac glycosides and their specific receptors are serving now as tools in the hunt for the corresponding endodigins (8,9). Thus, on the one hand messenger-receptor systems serve as tools in the development of new drugs while on the other hand drugs known already for a long time may contribute to the discovery of the corresponding natural messengers via displacement studies on receptor preparations.

RECEPTOROLOGY ESSENTIAL TO LIFE SCIENCES

Receptors are as real as enzymes. They can be localized, visualized in tissues (receptor-histochemistry), determined quantitatively, tested in vitro and in vivo, and receptor preparations may even serve as test systems for drugs. The function of enzymes is to convert substrates to products. It are the receptors, however, which determine when, where and to what degree this occurs. It is the interaction between repressor and derepressor molecules and their specific receptor systems in the cell nucleus which are determinant for the enzymic equipment of the cells. Sites for allosteric activation or inactivation on enzymes, are also receptors involved in feed-back regulations. Receptorology is as real as enzymology and even more fundamental to the understanding of life processes.

From the point of view of organisation and the regulation it involves, living organisms can be regarded as complex networks of receptor-effector systems, usually consisting of cellular units, linked by messengers.

In short: no life without receptors. No life sciences without receptorology. No insight in pathology and clinical medicine without the concept of messengers and the corresponding receptors (2,10). As a consequence, the development of drugs, drug design, without consideration of receptors involved in the action - which in practice often implies necessity of receptor-binding studies - is no longer realistic.

RECEPTOR CHARACTERISATION

For quite a long time, regulation and integration of biological processes have been a main aspect of physiology. It should be realized, however, that physiologists use the term receptor to denote small organs in the sensory system that are able to detect, for instance, temperature and pressure changes, pain and taste. Here, the term sensor is to be preferred, it makes more sense and confusion is avoided.

Also, it is necessary to differentiate between receptors (specific receptors) involved in the induction of particular effects, and indifferent sites of binding that may be indicated as acceptors, silent receptors or sites of loss. The use of the term specific receptor requires the fulfilment of a number of criteria (11).

Table 1

Criteria for receptor characterisation

1) Saturability and reversibility of binding.
2) Specificity including stereospecificity of binding, which implies high affinities.
3) A correlation between tissue responsivity and receptor presence as observed by selective pharmacon binding or receptor visualisation (receptor-histochemistry).
4) Mutual displacement of agonists by their specific (competitive) antagonists and among pharmacologically related agonists or antagonists - with due attention for the frequent occurrence of multipotent antagonists that block different types of receptors (e.g. those of the α-adrenergic type and those for dopamine or histamine) and of agonists that activate different types of receptors (e.g. those for progesteron and corticoids).
5) Correlation between affinity in receptor binding and "activities" on isolated tissues or even in vivo - with due attention for the role of metabolic activation or inactivation and of pharmacokinetics in general and the possible occurrence of spare receptors.