

ALFRED BENZON SYMPOSIUM 26

# NMR Spectroscopy in Drug Research

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## NMR Spectroscopy in Drug Research

# NMR SPECTROSCOPY IN DRUG RESEARCH

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EDITED BY

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# Preface

During the last decades, much has changed in the way in which drug development is approached. With increasing understanding of the structure of living matter, more emphasis can be placed on the design of drugs on a rational basis, taking advantage of our growing knowledge of molecular processes in healthy and diseased organisms. Drug research thus becomes increasingly dependent on such disciplines as structural chemistry, biochemistry and biophysics, molecular biology, enzymology and cellular biochemistry, and it benefits from the research tools used in these disciplines.

In the same period, revolutionary changes have taken place in nuclear magnetic resonance spectroscopy. The change from continuous irradiation to pulse techniques resulted in a dramatic increase of sensitivity, and development of multi-pulse methods permitted experiments beyond the imagination of scientists in the past. The technology of superconductors made it possible to construct cryomagnets with field-strengths an order of magnitude greater than those obtainable with iron electromagnets. Computers have been the third boost to NMR, allowing sophisticated control of experiments, extensive data processing, the advent of space-resolved spectroscopy, etc. This development still proceeds at a breathtaking pace.

Consequently, NMR spectroscopy not only strengthened its position as an indispensable tool for studies of the structure and dynamics of small and medium-size molecules in solution, but became a very important tool in biochemistry and biophysics. Along with X-ray crystallography and computer-based modelling methods, NMR is gaining rapidly increasing importance in drug design by receptor fit. The ability of electromagnetic radiation in the MHz range to penetrate into biological material places NMR in the forefront as a very promising tool for studies of biochemical and pharmacological processes in intact cells and tissues. Here, it is crucial that most elements can be chosen for monitoring at the molecular level. Being increasingly capable of providing quantifiable biological data, NMR spectroscopy offers great opportunities in drug-related research.

The aim of this symposium was to present a broad illustration of the appli-

cations of NMR to drug-related problems. Topics range from well-established applications within synthetic medicinal chemistry, through conformational analysis, intermolecular interactions, and structure and function of biomolecules, to drug metabolism and NMR studies of cells and tissues. Although not all of the topics described deal with drugs directly, they are believed to represent important prototype approaches of potential interest to drug researchers interested in physical methods. It is thus our hope that this volume gives an overview of the achievements and prospects of NMR spectroscopy in this area, and that it will provide inspiration for further integration of modern NMR methods into drug-related research.

*J. W. Jaroszewski, K. Schaumburg, H. Kofod*

# General Principles and Problems of Rational Drug Design

*Peter Goodford*

All of us have an idea about the traditional ways in which drugs are discovered. They were originally selected for their dramatic or symbolic properties: belladonna, arsenicals and gold preparations all come to mind, and Gerard comments on the shriek, which could initiate madness or death, uttered by the Mandrake root as it was wrenched from the ground. In those early days, of course, drug preparations having genuine therapeutic properties were rare, and therapeutic practice relied upon mixtures of relatively innocuous ingredients. Nevertheless, careful observation identified certain potent materials whose biological effects were specific and well-characterized; belladonna was one of these, as were opium, coca and digitalis.

## *Bioassay procedures*

A key problem in the discovery and development of therapeutic agents may at once be identified in this early work. It is necessary to have a reproducible, specific and straightforward bioassay, which must be directly relevant to the human target disease, if effective drugs are to be discovered. Each of these requirements should be considered in a little more detail.

Reproducibility is needed because the course of drug discovery is slow, and it is highly desirable that results obtained in 1987 should be comparable with observations in 1977. A common practice has therefore been to establish biological standards, which may be used world-wide to calibrate bioassays, and a bureau for this purpose was established in the U.K. during the early 20th Century. However, the use of standards may not completely eliminate errors due to biological variation, and irreproducible assay procedures are still the cause of much unsuccessful pharmacological research.

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Specificity is needed because similar end results may be observed biologically, although those results were induced by quite different mechanisms at the cellular or sub-cellular level. Perhaps the best-known example of this is provided by the study of muscle-relaxing agents. Tubocurarine was a South American arrow poison whose chemical structure was determined in the 1930's. Two nitrogen groups were present in the molecule, and a set of much simpler molecules of the general type:



were therefore studied. The molecule with  $n = 10$  was a powerful muscle-relaxant, but this satisfactory finding did not justify the very reasonable logic behind the research, because further work showed that the new compounds and the original tubocurarine worked by quite different mechanisms. Furthermore, as a Parthian Shot from the fates, more recent work has demonstrated that the original tubocurarine structure was itself in error!

A bioassay must be straightforward because this is an economic requirement when many thousands or tens of thousands of biological measurements must be made during the development of a single drug. If the procedure is too complex and time-consuming, there may be a temptation to take short cuts and thus invalidate the results. However, the requirements for specificity and straightforwardness are not mutually compatible. For example, the effluent liquid from a biosystem may flow over three successive isolated organs (e.g. a perfused heart; a piece of stomach muscle, and a strip of tissue dissected from the bronchial lining of the lungs) one after the other, in a single bioassay, and the differences between the three responses might be used as a measurement of specific biological activity. Techniques such as these represent the highest level of the pharmacologist's art, but they are not straightforward or cheap when fully costed, and it is not always easy to maintain reproducibility.

The final important requirement is that the bioassay should relate to the target disease. This is, for example, easily fulfilled when antibacterial agents are being studied, because a compound which cures an infectious disease in an animal model has a relatively good chance of working in the human. That is why we have so many antibiotics available today. On the other hand, it is not easy to devise an animal model for Alzheimer's disease, which is a poorly characterized failure of central cognitive functions in the elderly, and the lack of relevant bioassays can be a major problem when research on such disorders is undertaken.

### *Research and development*

The requirement for good bioassays has been considered in some detail for two reasons. First, because they are needed from the very start of the drug discovery program, and second because it is not always appreciated that they are such a crucial requirement. Moreover, NMR methods could be more widely applied in this area than they have been in the past.

The drug development phase includes the development of manufacturing methods for the large-scale production of pure material from freely available raw materials. It includes the development of assays for purity; of stability studies under a very wide range of environmental conditions; the selection of suitable packaging materials; and the choice of delivery methods so that the compound can be safely given to the patient at appropriate times and at an appropriate dose, while ensuring that the patient cannot abuse the compound. It includes the establishment of patent rights world-wide, and calls for centers of excellence to be funded so that every aspect of the compound's properties can be thoroughly evaluated. For example a detailed analysis of drug uptake; of drug distribution in the body; of drug metabolism; and finally of the excretion of both the drug and its metabolites must be made, and all this may need repeating in black, white and yellow men, women and children. It also includes clinical studies on a few healthy volunteers at first, and then a growing number of patients until the new compound can be generally released for more widespread clinical use.

The incidence of side-effects must then be monitored, because some of these can be very rare indeed, and will not be detected during the early clinical trials. Consider a compound which has an aberrant adverse action that only affects, on average, 1 person in 100 000 but then kills that 1 sensitive patient. The preliminary human studies might be made on a few dozen healthy volunteers and a similar number of patients. More extensive clinical trials would extend to hundreds and then a few thousand patients, at which time the compound might be released for general use in one country, perhaps the United Kingdom. In any one year there might be a million British patients who would benefit from the new drug, but of course some time would elapse before its therapeutic role was established, and only 50 000 might be treated in year one. Perhaps 1 patient might suffer from the toxic effects, but it would be impossible to draw any firm conclusion from such a chance event, even if the association with drug-taking were recorded.

Like many biological phenomena, however, the introduction of a new therapeutic agent develops exponentially, and 3 or 4 years later the compound might

be widely used in Britain, Continental Europe, the United States and Japan. Perhaps 10 million patients a year would receive it and, on average, 100 would then die from the "rare" toxic effect each year. Thus an apparently negligible and undetectable adverse reaction would become a totally unacceptable toxic effect, simply because the numbers of patients at risk from a new therapeutic agent might be so large.

It may be seen from this example that the development stage is never finished, but there must be constant monitoring for adverse effects and continuing development of new delivery methods. The use of the compound in a wider range of diseases must also be explored, and better compounds will be brought forward in order to combat problems associated with the original compound, and secure ongoing patent protection for the Company which made the initial investment, and thus introduced the novel therapy.

NMR plays a key role in many of these development activities, as we shall doubtless hear in the days ahead. However, I would like to place more emphasis on the *research* which always precedes the development stage, and ask what NMR can do for drug research as well as development.

#### *NMR in drug research*

There have been three traditional approaches to drug discovery. The first relies on Serendipitous observations, such as the chance discovery by an English country doctor that the local people of the village ate foxglove leaves as a cure for dropsy, or the finding that the condition of a weak heart would be improved if the patient contracted malaria and was then treated with quinine. Medicine will continue to benefit from acute observations of this type, but it is not easy to plan for them.

The second traditional method is by mass-observation. Many novel compounds are studied in many carefully selected biological assays, and one or two compounds are selected for further detailed study. This has always been a soul-destroying activity, because the chances of success are so small, but from the view-point of a pharmaceutical company it is cheap and easy to manage. Moreover it has always worked and continues to do so, and the odds of success increase with the size of the operation. An investment in 10 compounds and 10 bioassay systems gives 100 chances of success. By doubling the investment to 20 compounds and 20 assays one has 400 chances. Perhaps there might be a role for NMR here, since it does in principle allow so many variables to be monitored simultaneously.

The third traditional method is the detailed study of biological mechanisms, and to this must be added the relatively recent study of biological structures at the macromolecular level. Both these methods are leading to novel therapeutic approaches, and I hope that we shall hear much about the role of NMR in this context during the days ahead. By throwing light upon the dynamic properties of macromolecules, NMR is advancing our ideas about structure-function relationships in health and disease.

To take an overall perspective, traditional approaches started from the disease and searched for the drug. Today we are starting from the known structure and function of the biosystem, and by unravelling its detailed workings in health and disease we are finding new approaches to therapy.

The work of my colleagues at the Wellcome Research Laboratories demonstrates the new strategy. The physiology and biochemistry of oxygen transport had already been studied for upwards of 50 yr when I first became interested in hemoglobin (Goodford 1973). We decided to design novel therapeutic agents which would react specifically with the hemoglobin tetramer, and thereby modify its properties and cure disease. It took 3 yr to establish guidelines for the new approach (Goodford 1976), and it was not until 1984 that the structure of a specific candidate drug could be published (Beddell *et al.* 1984). At the present time clinical trials are still in progress.

I mention this work for four reasons. First, it shows that drug design by the method of receptor fitting is now a practical possibility (Goodford 1984, 1985, Hol 1986). Second, it demonstrates the slow time-scale of therapeutic research, and the need for the Governments and people of the world to encourage their pharmaceutical industries, if the discovery of drugs is not to grind to a halt. Third, it provides an illustration of effective collaboration between the rival approaches of the X-ray crystallographer and the NMR scientist, because our first compounds were designed from the known X-ray structure of hemoglobin, but we were unable to confirm how they bound to the macromolecule by X-ray methods. And fourth, it also demonstrates a collaboration between university and industry, because our NMR colleagues at Wellcome were unable to study such a large macromolecule as hemoglobin in the middle 1970's but meaningful results were obtained at Oxford, and I published my first and last NMR paper in collaboration with the Oxford group (Brown & Goodford 1977).

The study of structure and function at the macromolecular level has opened a new phase in the search for better therapeutic agents, and promises the introduction of more specific, more potent and safer drugs. NMR observations will play



a key role in this new and exciting advance, and the 26th Alfred Benzon Symposium comes at a most suitable time.

## REFERENCES

- Beddell, C., Goodford, P. J., Kneen, G., White, R. D., Wilkinson, S. & Wootton, R. (1984) *Br. J. Pharmacol.* 82, 397-407.
- Brown, F. & Goodford, P. J. (1977) *Br. J. Pharmacol.* 60, 337-41.
- Goodford, P. J. (1973) Concordance. In: *Biological Horizons in Surface Science*, eds Prince, L. M. & Sears, D. F. pp 427-448. Academic Press, New York.
- Goodford, P. J. (1976) The Haemoglobin Molecule as a Model Drug Receptor. In: *Drug Action at the Molecular Level*, ed: Roberts, G. C. K. pp 109-126. Macmillan, London.
- Goodford, P. J. (1984) Drug Design by the Method of Receptor Fit. *J. Med. Chem.* 27, 557-64.
- Goodford, P. J. (1985) A Computational Procedure for Determining Energetically Favorable Binding Sites on Biologically Important Macromolecules. *J. Med. Chem.* 28, 849-57.
- Hol, W. G. J. (1986) Protein Crystallography and Computer Graphics - toward Rational Drug Design. *Angewandte Chemie (English Edition)* 25, 767-78.