

# Green College Lectures

## Pharmacology: analysis and exploration

J W BLACK

Professor Desmond Laurence describes the domain of pharmacology thus: pharmacology is about drugs and drugs are about sick people. This definition is attractive, but it obscures the problem faced by pharmacologists—the problem of identity. Clinical pharmacologists see themselves as physicians with a special interest, but no physician can avoid having a special interest in drugs. Non-clinical pharmacologists are usually difficult to distinguish from biophysicists, biochemists, cell biologists, and so on. Indeed, every biomedical investigator or practitioner can claim to have an interest in drugs.

Some years ago Sir William Paton identified the characteristic achievements of pharmacology as follows: (a) pharmacological inductions about physiology based both on patterns of bodily action and on patterns of chemical structure; (b) bioassay for detecting and measuring minute amounts of active substances; (c) biostatistics for handling variable responses and estimating error reliably; (d) development of the principles of receptor function; (e) the introduction of valid methods of clinical trial.

From this list we can derive the general proposition that pharmacology studies the interactions between chemicals and biosystems. Specifically, pharmacology relates the properties of chemicals—chemical structures, physical characters, concentrations—to the perturbations or effects they produce in biosystems. Information flows in both directions between chemicals and the biosystems exposed to them. Knowledge about the properties and function of the perturbed systems transfers to the chemical by way of classification; knowledge about the properties of the chemical can be used to expose and explore our ignorance about the biosystem. Even if we knew virtually all there was to be known about biosystems, however, pharmacology would still be alive and well. The synthesis of new chemicals is entirely open ended, and each new chemical would invite analysis by an appropriate biosystem to measure and classify its biological properties with the aim of finding more efficient and more selective controlling devices. The centre of gravity of the pharmacological domain must therefore be closer to an inquiry about the properties of chemicals than about the nature of biosystems.

### Complexity and selectivity

The essence of biosystems is complexity, both structural and functional; correspondingly, the essence of pharmacology is selectivity—that is, non-uniform actions on complex biosystems. Chemical actions on biosystems can be studied at two main levels. Because chemicals must interact with chemicals their actions can be studied at the biochemical level. Chemical actions in turn lead to changes in tissues, organs, or animals so that they can also be studied at the physiological level. The effects of chemicals at the physiological level can be described, using nominal or ordinal

scales, to express selectivity: for example, vasodilator, hypoglycaemic, anti-inflammatory. Descriptive pharmacology is the heart of therapeutics based on the classification of consequences found in standard textbooks. At the biochemical level chemical interactions can be specified and measured using interval and ratio scales, which may provide a molecular analytical account of a chemical's selectivity. Where analytical pharmacology can specify such an interaction we have a tool which may be useful for physiological or clinical exploration. Exploratory pharmacology therefore depends on a classification based on mechanisms, a classification which has to be generated by analytical pharmacology. I argue, then, that analytical pharmacology can usefully be distinguished from descriptive and exploratory pharmacology, that it lies close to the heart of the domain of pharmacology, and that this, in turn, places pharmacology closer to physical chemistry than to physiology or biochemistry. I argue that by serving to focus attention on certain areas of interest this semantic conclusion has also got heuristic value. I will now try to do this by developing some ideas which are fundamental to pharmacological analysis.

### Pharmacological analysis

Pharmacological analysis presupposes that if order, pattern, and sequence are found at the physiological level when foreign substances act on biosystems this will correspond to some related orderliness at the biochemical level. At the biochemical level a vast array of proteins continuously participate in a vital network of mainly bimolecular reactions. In addition to the obvious structural role of proteins, four major functional classes of proteins are recognised: protein acceptors store specific ligands; carriers transport solutes across cell membranes; enzymes convert substrates into new molecular species; and membrane receptors transduce hormone binding into cellular responses. Directly or indirectly, acceptors, carriers, enzymes, and receptors turn out to be the elective sites where foreign chemicals also act. The biochemical systems behave as though the hormones, substrates, and so forth constituted an enormous internal pharmacopoeia. Today, biochemists are frenetically busy extracting and isolating all of these various sites as pure proteins. In addition to the previously extensive purification of acceptors and enzymes, biochemists have now started to purify and characterise the oligomers, which form not only membrane ion channels but hormone receptors as well. Once isolated, these proteins can bind, catalyse, create channels in artificial membranes, and activate various kinase transducers. Here, then, is the ultimate level at which pharmacological analysis might determine how and where foreign chemicals act.

But surely, at this level, when the interaction between pure substances is being studied, we have moved out of the pharmacological and into the chemical domain. Selectivity, the heart of pharmacology, cannot be expressed at this level. Even at a higher level of complexity, when tissue homogenisation has destroyed physiological activity but retained all the biochemical components and systems, selectivity of drug action can be expressed but it cannot be extrapolated to intact tissues. Indeed, hormone actions can be recognised, by definition, only in intact cells or tissues. The

gap between biochemical and physiological knowledge is unknown. In complex hierarchical systems events at a lower level have to be interpreted in terms of events at a higher level and not the other way round. Pharmacological analysis must therefore begin at a physiological level of cellular organisation. Which level should be chosen?

### Gastric acid secretion

Let me illustrate this point with an example. Imagine you want to study analytically the effects of gastrin, acetylcholine, and histamine on the process of gastric acid secretion. You could examine oxyntic cell function at several levels of complexity. The classic studies of human acid secretion via gastric fistulas on the stomach wall, the highest level, were Beaumont's observations on Alexis St Martin and the Wolffs' studies on Tom. Both subjects showed that stomach vascularity and acid secretion were integrated into their social and psychological lives. Their stomachs were affected not only by appetite and hunger but by anger and hostility as well. As Tom's face reddened with anger (which was quite often) his stomach mucosa became red and engorged at the same time. Plainly, at this, the highest level of organisation and regulation between the secretion of acid and other functions, sheer complexity would challenge the descriptive pharmacologist and confound his analytical colleague. Traditionally, therefore, acid secretion has been studied in animals with mucosal pouches to avoid contamination with food, and usually the stomachs were denervated (the Heidenhain pouch) to eliminate psychic influences. In the past 10 years or so, however, techniques for studying acid secretion *in vitro* have been developed. The lumen of the isolated mouse stomach can be perfused to harvest secreted acid.<sup>2</sup> This preparation retains an intact epithelium, subepithelial nerves, and mast cells and only an effective capillary circulation is missing (a great disadvantage in this case). The epithelium can be removed at the price of disrupting subepithelial structures, but the epithelial cells are properly polarised and secrete into the chamber of a perfusion cell. Suspensions of glands can be made so that the acid is secreted into the lumen and detected by indicator dyes. The oxyntic cells themselves can be isolated into a suspension and, at the lowest level, the cell can be fragmented for studying receptor coupled adenylate cyclase activity. This array of preparations shows decreasing physiological regulation as you move down the hierarchical scale, but moving up from the molecular level of receptors and enzymes each higher level shows increasing degrees of biochemical organisation. In general, pharmacological actions can be confounded by too much physiological regulation or too little biochemical organisation. The isolated mammalian stomach is a compromise between these problems. This preparation can be regarded as the physiological unit of acid secretion—that is, it has all the necessary elements to express unregulated secretory activity. Only at this level do all three physiological agonists—gastrin, vagal acetylcholine, and histamine—operate. Disorganise the gut epithelium and gastrin and acetylcholine cease to be effective; but histamine is still active in broken cell preparations.

I think this analysis can be generalised. I recommend that the tactics for analytical pharmacology are, firstly, to identify the appropriate physiological unit (which may vary in complexity from separate cells to intact organs) to establish major pharmacological actions—that is, selectivity—and to develop operational models of specificity. Then we can go to higher levels of organisation for a more comprehensive assessment of selectivity or to lower levels to explore hypotheses about specificity. The problem then comes down to this: how can we extract chemical information out of the behaviour of a physiological system?

### Relations between concentration and effect

A pharmacologist intent on using a physiological unit for analysis has to face the fact that there is not much he can do with it. The main variable he can control is the dose or concentration of the ligands which he has chosen to use. A lot of pharmacology is comparative,

and, unfortunately, a fairly common tactic is to compare responses to equimolar doses. This procedure economises on the number of measurements that need to be made, but the method assumes that, in making comparisons, the underlying shape of the dose-response curves is not altered. As the method prevents the testing of that assumption it is inherently unreliable. Pharmacological published work is peppered with examples of erroneous conclusions being reached by failure to apply this test. On the other hand, valid comparisons provide the engine for pharmacological analysis.

A satisfactory experimental basis for comparison was worked out many years ago when the analytical dilution assay was being developed to allow physiological systems to be used for measuring concentrations. In that case the comparison entails studying the same chemical under *different* circumstances. Provided they reach similar criteria of comparability, *different* substances can also be compared under *similar* circumstances. These comparative bioassays are the basis for studying relations between structure and activity and the industrial process of screening new compounds. Even under the best circumstances, however, while they may generate useful, interesting, or even surprising results they cannot unaided derive theoretically relevant chemical data. For example, in the original paper which proposed burimamide as a specific ligand for histamine  $H_2$ -receptors the pattern of histamine related responses produced by comparative bioassays was critical.<sup>3</sup> The relative agonist activities of three compounds—4-methylhistamine, 2-methylhistamine, and the 1,2,4-triazole analogue of histamine—were estimated relative to histamine on five bioassay systems. The methyl derivatives saw differences among them but the triazole analogue did not. The original paper referred to the differing groups as  $H_1$ , which had been chemically characterised by Ash and Schild,<sup>4</sup> and non- $H_1$ , which had still to be characterised. These assays did not do that. They did, however, produce a surprise—namely, that 4-methylhistamine was nearly half as active as histamine as a stimulant of acid secretion in the guinea pig but, comparatively, nearly inactive as a stimulant of visceral muscle in the same species.

Experimenting would be boring if we never got surprises. We get surprises when we find something we did not expect. Expectation means that we had a rule or an idea or a model in our heads before we started. Our prior conception deals with a surprise by accommodation or adjustment, usually by muttering something such as: "X is behaving as though Y had happened." Whenever we use such a simile to describe experience we usually express what we have said by some kind of mathematical equivalent—that is, an operational model. Making models forces us to shed woolliness in our thinking. In this form the idea is easier to challenge and to test experimentally. The strategy is to estimate model parameters, which have chemical meaning, from estimations of assay parameters, which do not have chemical meaning. Measurement of the full range of the concentration-response curves to get estimates of their parameters—that is, location, slope, asymptote—is therefore needed. More importantly, however, the ability of bioassays to determine chemical information depends entirely on the quality of the models we use to organise our thought and design our experiments.

### Pharmacological modelling

When chemicals are studied at the physiological level we are accustomed to lumping almost everything that happens between delivering the drug to the system and its response into a black box. But year by year our biochemical colleagues are hammering away at the black box letting in more and more light. Sooner or later, pharmacologists will have to look at their traditional models to see if, and if so how far, they can adapt to the new knowledge to exploit analytical opportunities.

The aim of pharmacological modelling is to define the chemical constraints—for example, dissociation constants—which characterise the interaction of hormones or other chemicals with biosystems and then to try to relate these model parameters to the descriptive parameters of the physiological responses. Pharmacological modelling is ultimately solved algebraically and then the solution can be displayed graphically; these are activities for mathematicians. The

modelling begins, however, by defining the elements to be modelled and their logical relation to each other. These elements come from pharmacological experience. Identifying the initial shape of the problem is therefore a headache for pharmacologists not mathematicians. The principles of the most elementary analyses can be presented as follows.<sup>5</sup>

The analysis of simple, competitive, antagonism . . . is also a form of dilution assay. Unlike the analytical dilution assay, however, assumptions about the nature of the actions of agonist and antagonist are needed. Both substances must be assumed to interact with common tissue components, receptors, and the interactions are assumed to be governed by the law of mass action: that is, their concentrations determine the fraction of receptors occupied, on average, at equilibrium. Essentially, the assay uses the agonist to measure the extent to which the cellular receptors are effectively diluted by the presence of antagonist. This chemical measurement is achievable because the use of ratios of agonist concentrations allows the post-receptor, physiological events (assumed to be constant for a single agonist) to be cancelled out. The assumption of the law of mass action allows the antagonist-receptor interaction to be characterized by a single binding parameter. By definition this statistic incorporates information about both the antagonist and receptor molecules and, therefore, when an antagonist is found to have significantly different parameter values on two physiological systems differences in the receptors must be inferred. This is the basic theorem in receptor classification.

As in other branches of science, the development of operational models is a necessary precondition for attacking complex problems. The models do not simplify but they can surely clarify the problems. The problems, after all, are enormous. There must be an axiom in pharmacology that no molecule can be assumed to have a single site of action in a complex biosystem because we cannot prove the converse and because evidence that molecules do have plural actions is common. We have to develop pharmacological strategies to match. We could begin by removing a handicap which we have made for ourselves. In our textbooks and professional journals pharmacologists have so far reached no agreement about nomenclature, notation, definitions, or criteria. The disturbing feature of the present position is not so much that there is chaos or even apathy but what seems to me to be a positive lack of enthusiasm about the need to bring discipline into our discourse and our thinking. Fortunately, the impact of biochemistry on the traditional domains of pharmacology is now becoming so strong that reform should be on the way in spite of ourselves.

Pharmacology is unique in the biomedical sciences in having an inexhaustible supply of new molecules to play with. So there is much interest in pharmacological circles in the way that new molecules produce novel effects in biosystems. What is much more interesting, however, is to find that a number of quite different molecules can be shown to have the same effect—that is, they belong to the same class. Classification, I believe, is about the future of pharmacology. It identifies anomalies, feeds the imagination, and assembles valid targets for medicinal chemists to aim at.

### Pharmacological classification

An analytical pharmacologist can conclude only that chemical A is behaving in a particular biosystem as though it interacted with chemical components B and C under circumstances where B and C are already defined and classified. So analytical pharmacological classification is contingent on biochemical classification. Fortunately, biochemists have been developing their own systems of classification for nearly 50 years. Biochemists surveying the contemporary scene in pharmacology must surely have a sense of déjà vu. Here are some extracts from the opening paragraphs of *Enzyme Nomenclature*: "The rapid growth in the science of enzymology, and the great increase in the number of enzymes known have given rise to many difficulties . . . By about 1955 it had become evident that the nomenclature was getting out of hand . . . Moreover . . . in the equations of enzyme kinetics different systems of mathematical symbols were in use and the standardisation of enzymes was in a chaotic state owing to the multiplicity of arbitrarily defined units."

An international commission of enzymes was set up in 1955 and has reported at regular intervals since 1961. Each enzyme now has a systematic name which specifies the physiological substrate and its catalytic fate; there is a trivial name for ease of communication and a numerical code setting out its hierarchical class. By establishing the principles of classification the commission also established the standards and criteria to be met before a new enzyme is classified; if there is inadequate evidence there is no classification. Analytical pharmacology has, I believe, been a major beneficiary of all of this. The classification of ligands and drugs as enzyme substrates or inhibitors is about the only area of analytical pharmacology which is not in a mess.

The major problems arise in relation to hormone receptors, a large class of specific proteins. To begin with there is no agreement on how we should define or restrict the use of the word hormone. Do neurotransmitters have features in common with steroids which allow them both to be classified as hormones? That might simply be a semantic question. Should cotransmitter peptides in the brain be classified as neurotransmitters or hormones? That might well be a conceptual question. Again, there is no agreement anywhere about how to use the word receptor. I have no doubt now that receptor is a meaningless noun; only the prefixes: hormone, mechano-, and so on make sense. Nevertheless, even if we all agreed on how we should use these words and ideas the problems would still be immense. Hormones, by definition, can be recognised only by changes in the behaviour of cells or tissues—the physiological domain. At present hormone receptors can be provisionally defined only by the interaction of efficacy-free ligands with the native hormone-effect biosystem—that is, the pharmacological domain. Hormones and their receptors provide the physiological route for posting commands at the biochemical level and determining the likelihood of particular outcomes at the behavioural-physiological level; therefore drugs—agonists, partial agonists, antagonists—interacting with hormone receptors offer the industrial drug researcher the most predictable outcomes at the selectivity-therapeutic level—this is the medicinal chemist's domain. Very high affinity or covalent binding ligands, which are pharmacologically well classified, are essential for attempts to extract, purify, and characterise hormone receptors—the biochemical domain.

Plainly, in all these aspects of receptor research pharmacologists are in a central position. The ligands they classify are the key to all the rest. And this is where we get into a pickle. Pharmacologists have not agreed to enforce the criteria necessary to conclude that a particular receptor paradigm is incompatible with the new evidence and must be replaced. There seems to be no feeling for the possibility that of all the explanations for ligand or drug related phenomena differences or subclasses of receptors might be the least likely. The combination of plural actions of chemicals and multiple tissue recognition of chemicals and multiple tissue recognition sites can generate a wide variety of responses. The other problem is the lack of agreement on the information needed to identify a new receptor. In classifying enzymes the Enzyme Commission apparently tried to use as much information as possible. By analogy, we might therefore expect that in the classification of hormone receptors we would want to know: (a) the native hormone; (b) the evidence of subclasses, cofactors, and so on; and (c) the proximate change or transducer. As with the Enzyme Commission's treatment of tissue species variations, these would also be invaluable in footnotes to a hormone receptor taxonomy. Despite this, a recent conference on receptor classification was repeatedly advised by leading pharmacologists that binding information about foreign ligands was not only necessary but sufficient for hormone receptor classification. The problem is not trivial. At one extreme, I think there would be intellectual satisfaction if we really tried to identify the conjugate hormone—for example, it might be that instead of  $\alpha_1$ ,  $\alpha_2$ ,  $\beta_1$ ,  $\beta_2$  receptors we ended up with adrenaline  $\alpha$  and  $\beta$  receptors and noradrenaline  $\alpha$  and  $\beta$  receptors. At the other extreme, there would be a flood of discipline into pharmaceutical research so that investigators would give up the wishful thinking exercise of seeking what they would like to have and concentrate on what evidence and theory indicated they were entitled to expect.

In one sense, the contribution which pharmacologists might

make to the classification of hormone receptors should be the least of their worries. The larger problem is how to classify multivalent ligands and drugs. Some kind of non-Linnean, numerical taxonomy may be needed. The classification at this level is not, of course, for clinicians and therapists. It is the exploratory pharmacologists whose needs are greatest. Schild recognised 40 years ago that several antagonists, like an image intensifier, had to be superimposed to give reliable information. I have always been cautious about concluding that because a drug, such as burimamide, competitively antagonised histamine and also antagonised pentagastrin the latter action was a consequence of the former. On the other hand, when 20 or more quite different chemicals with different physical and pharmacological properties not only competitively antagonise the action of histamine on heart muscle but also block pentagastrin then there is indeed a strong inclination to conclude that one is due to the other. For me, a struggle with problems of classification is a major obligation for pharmacologists and the framework for new drug development.

### Exploratory pharmacology

Pharmacologically classified drugs can be used at two levels: to manipulate biosystems at the physiological level, which is their main use in therapeutics, and to probe biosystems at the biochemical level to uncover mechanisms and regulations. In the first case, the analytical classification is interesting but nearly irrelevant. In the second case, the analytical classification can be beguiling but misleading. There is no way that the analyst can assess that a compound has a specified action at the biochemical level and be able to add the rider "and it has no other actions." Even the use of several similarly classified ligands for investigation is not free from weakness. Of course, I am quite prejudiced by my experiences with using  $\beta$  receptor and  $H_2$  receptor antagonists for exploration, but I am inclined to think that when ligands are used for exploratory work the results must be handled with caution. For example, the original idea behind developing adrenaline  $\beta$  receptor antagonists was that cardiac inotropic activity due to adrenaline was metabolically expensive, its so called anoxiating action. The drugs proved to be useful in treating angina pectoris but the original idea has still to be tested—for example, by offsetting the negative inotropic and

cardiac dilational effects of the  $\beta$  receptor blockade by digitalis. Subsequently,  $\beta$  receptor antagonists were found to be effective antihypertensive agents, but after 20 years there is still no agreement on how they work or indeed about the role of  $\beta$  receptors in initiating and maintaining hypertension.

The idea behind the development of the histamine  $H_2$  receptor antagonists was that drugs with this property might be helpful in exploring the relations between gastrin and histamine.  $H_2$  receptor antagonists suppress acid secretion evoked by both gastrin and histamine. Nevertheless, 15 years after their discovery the relations between gastrin and histamine are still entirely speculative. I prefer the explicit model—that is, that gastrin acts on subepithelial mast cells to release histamine, which increases capillary flow ahead of the increase in acid secretion—but my preference for this over another model is largely aesthetic.

At the end of it all, we have put in a lot of effort and barely changed the balance of probabilities. I do not think my experience is either unique or even uncommon. When drugs are used therapeutically they are often being used to introduce bias into a regulated process. No great precision is needed and a resultant action may not only be adequate but may, indeed, be desirable. When the same substance is used as an exploring ligand, however, it may simply lack the authority because of imprecision, resultant activity recognised or unrecognised, or the lack of appropriate modelling. Drugs are neither physicians' scalpels nor biophysicists' lasers. As with much else in life, the usefulness of drugs is a function of the care and attention we pay to them.

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*A 32 year old woman's sister died of carcinoma of the breast aged 28 and two aunts developed breast cancer in their 40s. Does the benefit of mammography at her age outweigh the risk of it causing anxiety? Should I refer her now for screening or wait a few years?*

Carcinoma of the breast developing in a sister or mother unilaterally after the menopause represents only a negligible increase in the risk of breast cancer in the patient, perhaps  $1.2 \times$  the expected incidence. There is, however, an undoubted increase in the risk (perhaps two or three times normal) if the breast cancer occurred premenopausally and the risk is even greater in those unusual cases where the relative's tumour was bilateral. Actuarial figures are published giving the likely percentage incidence of breast cancer at different ages in sisters of patients with breast cancer.<sup>1</sup> In the present instance, this patient needs to be told that she is indeed at increased risk of developing this disease although she is much more likely never to develop breast cancer. She should be counselled that it would be a perfectly reasonable and worthwhile course for her to undergo regular screening, which is both simple and painless. She should be carefully examined clinically and taught, at that time, how to carry out breast self examination monthly. At the age of 32 it would be reasonable to carry out a base line mammogram and to repeat this every other year or even annually. The patient will obviously be anxious about her family history and if the facts and outline of screening are put to her these should allay rather than aggravate anxiety. Unfortunately, the accuracy of mammography is undoubtedly reduced in the premenopausal breast in which the breast tissue is usually dense and thus presents some difficulties to the radiologist.—HAROLD ELLIS, consultant surgeon, London.

1 King MC. Breast cancer genetics; definitions of risk, patterns of inheritance and management of patients and family members. In: Forbes JF, ed. *Breast disease*. Vol 10. *Clinical surgery international*. Edinburgh: Churchill Livingstone, 1986.

*What is the most reliable and cheapest urine pregnancy test you would advise for clinical use by general practitioners and pharmacists?*

The diagnosis of pregnancy is, like any other diagnosis, based on history and physical examination. Laboratory investigations may form a special part of the physical examination and may add useful information. This basic clinical truth has been overtaken by twentieth century folklore in which a pregnancy is not a pregnancy until it has been legitimised by a positive urine test. Accepting that such tests are likely to be performed in the settings envisaged by the questioner, we can consider the general characteristics that the chosen method should have. The specimens to be analysed should be appropriately collected (dilute urine will offer lower sensitivity—an early morning specimen will be best) and should be free from contamination and sediment. The test should be simple in performance and interpretation, should have adequate sensitivity to detect pregnancy at the stage when tests are to be performed, should not be susceptible to false negative results in circumstances of chorionic gonadotrophin excess (such as hydatidiform mole) and false positive results due to luteinising hormone, should have stable reagents with long storage life, and should be economical. These characteristics are likely to be provided better by an agglutination-inhibition test than by an agglutination test, and several suitable test kits are on the market. Enzyme linked immunosorbent (ELISA) tests that provide a coloured endpoint have recently become available. While they are rather more expensive, they may provide a useful increment in sensitivity and ease of performance. Whatever method is chosen, the tests should be performed by a properly trained and experienced operator who can provide a reliable analytical result. These conditions are often best met by formally referring the specimen to a chemical pathology laboratory for analysis.—D R BOSWELL, senior lecturer in chemical pathology, Southampton.