Gaddum's Pharmacology

Ninth Edition

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OXFORD · NEW YORK · TORONTO
OXFORD UNIVERSITY PRESS
1985

Oxford University Press, Walton Street, Oxford OX2 6DP
Oxford New York Toronto
Delhi Bombay Calcutta Madras Karachi
Kuala Lumpur Singapore Hong Kong Tokyo
Nairobi Dar es Salaam Cape Town
Melbourne Auckland
and associated companies in
Beirut Berlin Ibadan Nicosia

Oxford is a trade mark of Oxford University Press

Published in the United States
by Oxford University Press, New York

First Edition 1940
Ninth Edition 1985

British Library Cataloguing in Publication Data
Gaddum, Sir John
Gaddum's pharmacology. — 9th ed.
1. Drugs 2. Pharmacology
I. Title II. Burgen, Sir Arnold
III. Mitchell, J.F.
615'.1 RM300

ISBN 0-19-261423-1

Library of Congress Cataloging in Publication Data
Gaddum, J.H. (John Henry), 1900–1965.
Gaddum's Pharmacology.
Oxford medical publications)
Includes bibliographies and index.
1. Pharmacology. I. Burgen, A.S.V.
II. Mitchell, J.F. (James Fabian) III. Title.
IV. Title: Pharmacology. V. Series. [DNLM: 1. Pharmacology. QV 4 G123p]
RM300.G3 1985 615'.1 85–7097
ISBN 0-19-261423-1 (pbk.)

Set by Cambrian Typesetters, Frimley, Surrey
Printed in Great Britain by
St Edmundsbury Press
Bury St Edmunds, Suffolk

Preface to the ninth edition

The pace of development in pharmacology continues to be very rapid and, since the last edition was prepared, there have been fundamental advances in many areas that have altered or increased our knowledge of the subject in significant ways. For instance, studies on drug receptors have been a field of intense activity and we now see the beginning of the isolation, chemical characterization and even synthesis of these structures, which are so basic to our understanding of drug action.

Another area of real advance has been the studies of transmitters in the nervous system and this has produced important changes in our interpretation of the central actions of drugs. This is reflected in a major change in the presentation in this edition.

In many other areas our knowledge of how drugs act has increased dramatically over the past decade and, while this allows logical and clear explanations to be given of the way an increasing number of drugs work, it also means individual drugs and their related mechanisms are found to have importance in an increasing, and sometimes bewildering, variety of different systems and situations. To avoid unnecessary repetition we have described each drug or mechanism only once in detail but we have increased cross-referencing throughout the volume.

Another innovation is that we have been strict in describing drugs in the text only in their approved or chemical names but at the end of the volume will be found a comprehensive list of drug name alternatives including the most common proprietary names, with American and other alternatives given whenever appropriate.

The objective of the book has not changed, namely to act as an introduction to modern pharmacology for pre-clinical medical students and students of science and pharmacy, with emphasis on mechanisms of drug action and the relationship of the chemical structure of drugs to their action.

The book does not attempt to cover the clinical aspects of pharmacology but rather to provide a sound basis for the subsequent study of the use and actions of drugs in man.

December 1984

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J.F.M.

Contents

1. General pharmacology

What are drugs?

DRUGS are chemical substances which, by interacting with biological systems, are able to change them in some way. They may cause contraction of muscles, or secretion by glands, the release of hormones, or alterations in nervous activity, change the rate of division of cells, or actually kill cells. The variety of drug action is very great, and in principle it is possible to modify any biological process by drugs. Indeed, drugs have been found that affect almost every biological process.

In nature drug action is found in many inorganic substances (e.g. mercury, thallium, lithium, iodine, fluorine) and in various organic substances ranging up in size to large macromolecules (proteins, polysaccharides, polynucleotides).

The fact that drugs differ in their actions means that they must be selective in the biological structures with which they interact and, indeed, in the way that they affect different cells. The selective action of a drug depends on forming a combination with certain tissue components and not with others. As a rule this is a relative matter, i.e. the drug can combine with more than one tissue component but it binds more strongly to one component (and hence its effect is predominantly on the component to which it binds most strongly). In rare cases the selectivity of the drug is so great that we can call it **specific**, but this is very unusual and we must always be aware that a particular action of a drug may be due to one of these 'secondary' actions. Because of the relatively low specificity of drugs, we commonly speak of a **main action** – i.e. the one that we wish to use therapeutically or pharmacologically – and **side actions**, which are undesirable in the particular application. Serious side actions that have adverse effects on the subject and may even lead to death are **toxic actions**. Toxic actions may simply be a manifestation of overdose of the drug, but more frequently they are unrelated to the primary action of the drug and may either be one of the side actions or the result of metabolic transformation.

In earlier times, drugs were discovered from the finding that certain plant or mineral substances were toxic, for instance the beans of the cascara caused violent purging, or the berries of the deadly nightshade caused dilatation of the pupils, a bright red flush of the skin and convulsions. However, the old pharmacopoeias consisted mainly of plant and other preparations that were harmless and that have subsequently been found to have little pharmacological activity. Their use is to be attributed mainly to their supposed magical properties.

In the early nineteenth century pure drugs of plant origin were isolated (e.g. morphine from opium, atropine from belladonna) and with these, the scientific study of drugs could begin. Later in the nineteenth century with the development of systematic organic chemistry, entirely new compounds were discovered, many of which were found by empirical study to have interesting biological activity. Also the isolation of pure materials from animal tissues have shown the presence of natural regulators, hormones, neurotransmitters etc. that continue to provide new endogenously derived agents. All these substances can act as models that provide scope for the medicinal chemist to produce variants and analogues – a major way in which new drugs are developed. Natural sources of bacterial and fungal material have been the main way in which antibacterial and antitumour substances have been found (e.g. penicillin, streptomycin, vincablastine etc.).

Drug action on isolated tissues

Since the action of drugs in the body may be

complicated by many factors, including distribution, metabolism, and the interaction between different systems, it is easier to investigate some of the basic actions of drugs on a simplified system such as a piece of tissue isolated from the body. One of the simplest tissues to use in this way is the small intestine. The procedure for using it was first introduced by Magnus. If a short piece of small intestine (2–5 cm) is placed in a salt solution whose ionic composition is similar to that of blood plasma, and the solution is kept warm and oxygen is blown through it, then the gut will survive and respond to suitable drugs for many hours. Such a piece of gut can be set up so that one end is anchored and the other is connected to a lever so that the length of the intestine is recorded (Fig. 1.1). The length of

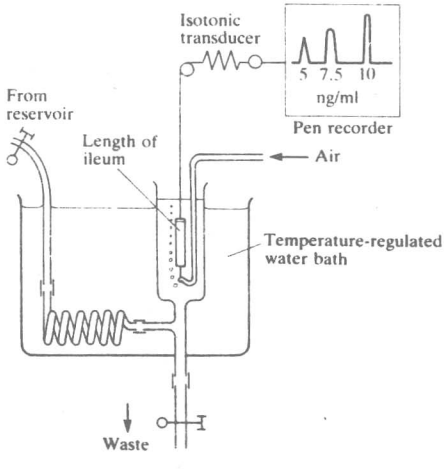


Fig. 1.1. Arrangements for recording the contractions of the longitudinal muscle of the small intestine. The water bath is usually kept at about 35°C.

the intestine is determined by the tone of the longitudinal muscle coat, so that the addition of substances that contract this muscle will be registered as a shortening of the piece of intestine. *In vivo* this muscle layer contracts in response to activity in nerve fibres originating in the myenteric plexus. These nerves release the chemical mediator, acetylcholine (Fig. 1.2) from their terminals and it is the acetylcholine that produces the contraction rather than any direct action of the nerves. By adding a solution of acetylcholine to the bath in which the intestine is immersed we can produce a similar response. On adding the

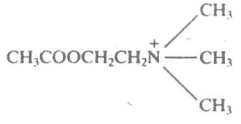


Fig. 1.2. Acetylcholine.

acetylcholine the muscle begins to contract within a few seconds. The muscle may be relaxed back to its original length by removing the bath fluid and replacing it by fresh fluid; the relaxation occurs rapidly and a further test may be made in a few minutes.

If we reduce the concentration of acetylcholine sufficiently we will eventually reach a concentration that is ineffective; if we systematically increase the dose from this level we will find that the degree of contraction increases with dose until a maximum is produced, and further increase in acetylcholine concentration does not produce a greater effect. The curve relating dose to response is called a **dose-response** curve and the type of curve obtained is shown in Fig. 1.3.

This graded response to drugs is the most common behaviour studied but there are, of course, cases where this cannot occur. For instance, if one is trying to find the dose of drug that kills an animal, the effect is quantal, that is, the animal either dies or survives and no intermediate category of 'half dead' exists.

Although acetylcholine is the natural excitant agent for the longitudinal muscle, it is not necessarily the case that the chemical structure of this substance is unique in being able to produce this action. If we look at the structure of acetylcholine we see that it is the ester of the quaternary ammonium base choline with acetic acid. One of the simplest and most informative ways of finding out how specific a chemical structure is needed for a biological action is by preparing a homologous series. This is done by modifying a particular chemical

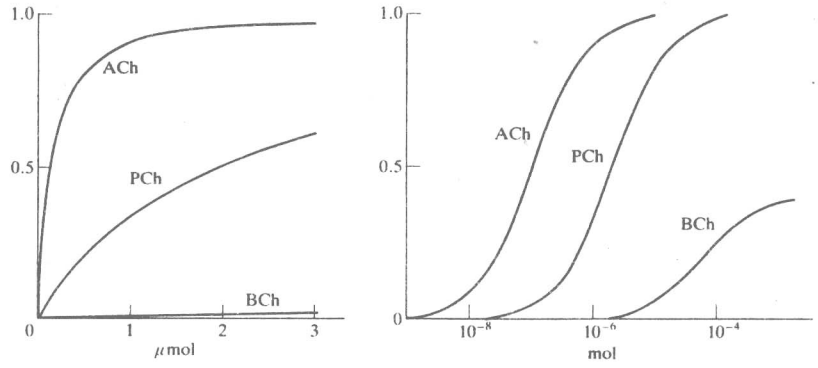


Fig. 1.3. Dose–response curves obtained on the guinea-pig ileum. The ordinate shows the contraction as a percentage of the maximum obtainable with acetylcholine (ACh). In the left hand graph the concentration of drug is shown on a *linear* scale; in the right hand graph it is shown on a *logarithmic* scale. PCh = propionylcholine; BCh = butyrylcholine.

group by the addition or removal of carbon atoms. In the present case we can do this by preparing esters of choline with the series of normal aliphatic acids – formic, acetic, propionic, butyric, valeric, etc. (Fig. 1.4).

Let us now test **propionylcholine** in the same way that we did acetylcholine on the intestine. It also produces a contraction that is graded with dose, and when sufficient propionylcholine is added to the bath a maximum contraction is produced that is exactly the same as that produced by acetylcholine. The form of the dose-response curve is shown in Fig. 1.3. The only difference is that about twenty times as much propionylcholine is needed to produce any level of response as is needed for acetylcholine. We can say that propionylcholine is a **full agonist** (an agonist is a drug producing a directly observable physiological effect; it is *full* because it produces the same maximum as a related drug) but its *potency* is 5 per cent of that of acetylcholine. If we now test butyrylcholine we find that it is about ten times weaker than propionylcholine and that the maximum response produced is not as great as that of acetylcholine or propionylcholine; it is therefore called a **partial agonist** and its potency is only 0.5 per cent of acetylcholine. The next number of the series, valerylcholine, produces no effect at all and is not an agonist. At the other end of the

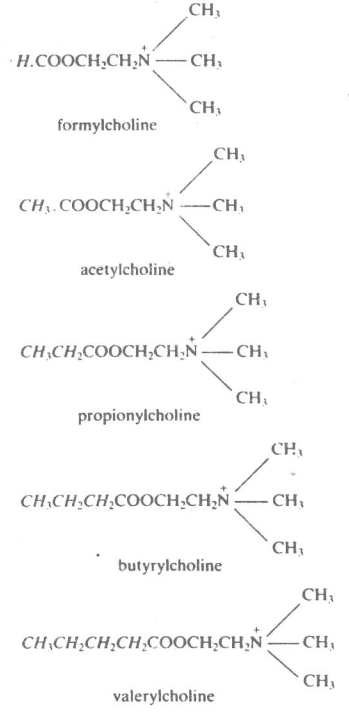


Fig. 1.4.

scale formylcholine is active, is a full agonist, and has about 25 per cent of the potency of acetylcholine.

We have learned from these experiments that acetylcholine is not unique amongst the normal aliphatic esters of choline in being able to contrast intestinal smooth muscle but that it is the *most potent* of the series. We find that small changes of chain length are tolerable but give reduced potency, and that lengthening by more than two carbon atoms leads to complete loss of ability to cause contraction.

Similar homologous changes can be made in the length of the alcohol group and in the methyl groups or the nitrogen. The results are similar i.e. acetylcholine is the optimal structure. Small changes can be made with retention of reduced activity, and larger changes cause loss of agonist activity.

We can next look at whether the particular chemical groupings in acetylcholine are essential for activity. Apart from the methylene and methyl groups, the acetylcholine molecule consists of a carbonyl, an ester oxygen, and the positively charged onium. In Table 1.1 the

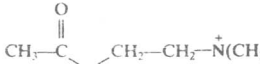
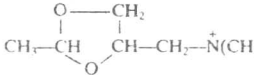
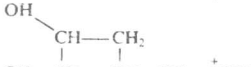
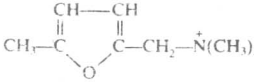
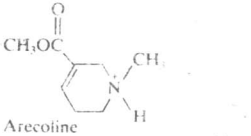
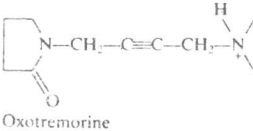
Table 1.1

	Relative activity
CH_3–CO–O–$CH_2CH_2N(CH_2)_3$ Acetylcholine	100
CH_2–CH_2–O–$CH_2CH_2N(CH_4)_3$ Choline ethylether	8
CH_3–CO–CH_2–$CH_2CH_2N(CH_3)_3$ 4-ketopentyl trimethylammonium	0.5
CH_3–CH_2–CH_2–$CH_2CH_2N(CH_3)_3$ Pentyl trimethylammonium	0.5
$CH_3N(CH_3)_3$ Tetramethylammonium	0.1

effects of changing some of these groups can be seen. Replacement of the carbonyl by a methylene reduces potency by a factor of twelve, the replacement of the ester oxygen has a larger effect. However, when the side chain is replaced by a normal paraffin with the same number of backbone atoms (n-pentyl), activity still remains, as it does even in the very simple compound tetramethylammonium.

Up to this point we have applied simple chemical reasoning to the study of a structure activity series, but new drugs are often found as a result of **screening**, i.e. new chemical compounds are tested empirically on a biological system to see if they have activity and by this means activity may be found in quite unexpected structures. Table 1.2 shows the

Table 1.2

	Relative activity
 Acetylcholine	100
 Cis-dioxolane	1000
 Muscarine	300
 Methyl furmethide	300
 Arecoline	100
 Oxotremorine	50

structure of five such compounds and in the case of three of them the activity is greater than that of acetylcholine. Muscarine and arecoline are natural compounds obtained from toadstools and betel nuts respectively. The other three compounds were prepared synthetically. The first three compounds show

obvious structural resemblances to acetylcholine: they all have a terminal methyl group connected through a carbon-oxygen-carbon linkage to a final $-CH_2N(CH_3)_3$. In the case of arecoline the resemblance is not nearly so obvious, and in the case of oxotremorine it is really rather remote. When the resemblance to the parent compound is so slight we must bear in mind the possibility that this drug is not acting by the same mechanism. We will return to this point later.

These observations have shown that the structure found in a natural agonist may not be uniquely necessary for the action it produces. A variety of chemical structures may retain the features that give the substance both selectivity and high activity. This fact is very generally true amongst drugs and really is the basis of much drug development. It gives scope to the synthetic chemist to produce variant structures with special properties differing in degree from that of the natural prototype. It is obvious that there must be some chemical features of drugs to which their actions can be ascribed, but discovering these is not at all straightforward and will be discussed later.

Dose–response curves

In Fig. 1.3 the dose–response curves for acetylcholine, propionylcholine and butyrylcholine have been plotted in two ways. In the left-hand panel the dose has been plotted along the abscissa on an arithmetical scale. The form of the curve is that of a rectangular hyperbola. In the case of acetylcholine the values corresponding to the main part of the curve are compressed at the left side of the graph.

On the other hand, with the less potent propionylcholine the main part of the curve is displayed but the upper values cannot be included. This is also the case for butyrylcholine. To the unpractised eye the resemblances between the shape of the two curves is not obvious.

In the right-hand curve of Fig. 1.3 the doses on the abscissa have been placed on a logarithmic scale. The effect is to turn the curve into a symmetric S-shape that accommodates all the values for acetylcholine. It is equally effective at accommodating all the values for propionylcholine and gives a curve identical in form but displaced to the right (i.e. to higher doses) because of its lower potency. It is now obvious that at all doses the potency of propionylcholine is lower than that of acetylcholine by a fixed ratio (20) corresponding to a 1.3 log units shift along the abscissa. We can refer to the curves as *parallel*. It is now also clear that butyrylcholine is not only even less potent than propionylcholine but it is not capable of giving a full response i.e. it is a partial agonist.

Log dose–response curves are almost invariably used for plotting the results of pharmacological measurements for the following reasons:

1. Most commonly, substances acting on the same biological system give curves of the same form; on a log scale this is easy to see, and the parallelism of the curves can be observed.
2. Ratios of potencies are easily estimated.
3. A logarithmic scale gives an equal weighting to all dose levels and so allows a wide range of doses to be plotted on a single graph without compression of any part of the curve. It allows accurate plotting of the responses to substances even when they differ in potency by a large ratio.
4. The middle part of the response range is very nearly linear. If responses between 20 per cent and 80 per cent of the maximum are obtained, a straight line may be drawn through these points; this is very useful when the curve must be defined from only two or three observations.

Antagonists

Up to this point we have taken as evidence of drug action a contraction of the longitudinal muscle which we have called an **agonist response**. We have not considered the possibility that the drug might be interacting with the tissue in such a way that it does not

produce an **observable response** directly but might be able to modify the response of the tissue to an agonist.

An example of this is shown in Fig. 1.5. The

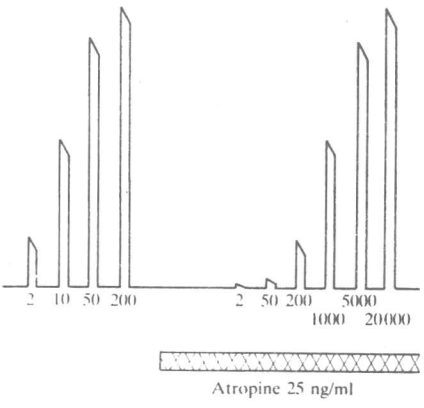


Fig. 1.5. The contractions of the ileum shown on the left part of the curve were produced by the concentrations of acetylcholine marked under each curve. After adding atropine, 25 ng/ml, to the fluid bathing the intestine larger doses of acetylcholine were required to produce effects equal to those produced before atropine was added.

dose–response relationship for acetylcholine was established and then atropine sulphate was added to the Ringer solution bathing the intestine. No direct effect was produced by atropine but when, after a few minutes, the response to acetylcholine was retested, the response to the lowest dose of acetylcholine was barely perceptible. However, when increased doses of acetylcholine were used, larger responses were obtained and indeed with a large enough dose a maximum response was obtainable that was not smaller than before atropine was applied. When the muscle was immersed again in Ringer solution containing no atropine the normal sensitivity to acetylcholine was gradually restored, i.e. the antagonism is reversible. If we look carefully at Fig. 1.5 we will see that to obtain matching sizes of responed, one hundred times the concentration of acetylcholine is needed in the presence of atropine. If we plot the log dose–response curve of acetylcholine in the presence of atropine it is shifted to the right in a parallel manner and the effect is to make acetylcholine appear to be one hundred times less potent as an agonist (Fig. 1.6). If a lower concentration of atropine is used, the shift to the right is less, and with a higher concentration the shift is greater; the degree of antagonism is thus dose dependent. The degree of antagonism can be conveniently characterized by the ratio of the dose of agonist needed to produce a matching response in the presence of the antagonist to that in its absence, this is referred to as the **dose ratio** (d); the rule governing the relationship of d to concentration of antagonist is that

$$(d-1) \propto \text{concentration of antagonist.}$$

It is well to note that with the antagonist action studied here, where the antagonism can be overcome by increased concentration of agonist, the antagonist action is often referred to as **competitive**.

The simplest explanation of the results with atropine is that, if acetylcholine combines with an 'acetylcholine receptor' to produce its action, then atropine combines with the same receptor *without* producing an action but denies access to the receptor by acetylcholine. The antagonistic effect of atropine is thus due to its ability to compete with acetylcholine for the acetylcholine receptor. Our earlier discussion of structure–activity relationships of acetylcholine congeners emphasized points of chemical resemblance, yet atropine at first

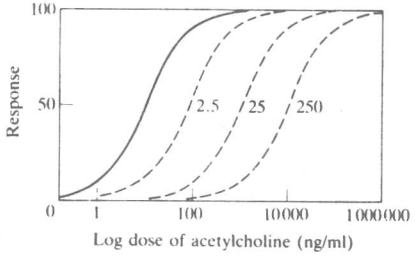


Fig. 1.6. Antagonism of acetylcholine by atropine on the isolated guinea-pig ileum. The solid curve is the dose–response curve in the absence of atropine. The hatched curves are the acetylcholine dose response curves in the presence of the concentration of atropine indicated (ng/ml).

sight (Fig. 1.7) has little resemblance to acetylcholine. Certainly there is an ester grouping and a basic nitrogen, but beyond that the similarity is less obvious. However, the hypothesis gains ground when we reconsider the longer-chain esters of choline of which valerycholine is an example. We noted earlier that this substance was not an agonist. However, in appropriate concentration it will reduce the response to acetylcholine and behaves just like atropine, although it is much less potent. In fact we find that it is a general rule that when the acyl part of acetylcholine is substituted by bulky residues, particularly aromatic groups, potent antagonists are produced; for instance, the choline ester of benzilic acid has about the same potency as atropine.

Since the resemblance to acetylcholine is more obvious in these compounds, it becomes more plausible that they might be combining with the same receptor.

It is apparent that this proposal also makes the assumption that atropine and related antagonists should not only antagonize the actions of acetylcholine itself but should also antagonize the actions of *all other agonists interacting with the same receptor*. This is indeed found to be the case and, for instance, it confirms that oxotremorine, in spite of its unusual chemical structure, also combines with this receptor. Indeed, with few exceptions the quantitative nature of the antagonism applies to all the agonists so that the *same dose ratio* is found for a given concentration of antagonist.

Suppose, however, that one of the agonists we had included in our structure–activity series was not producing contraction of the ileum by combining with the acetylcholine receptor but really belonged to a totally different class of drugs and reacted with a *different receptor*; in this case we would expect that an acetylcholine antagonist such as atropine would not antagonize it.

Among the substances that contract the smooth muscle of the guinea-pig ileum is histamine; it has a similar potency to acetylcholine, produces the same maximum response, and gives a similar log dose–response curve. Its structure (p. 43) is not very similar to acetylcholine, but we have learnt to treat this lack of similarity with some caution. Let us apply the antagonist test. The test procedure is shown in Fig. 1.8. Doses of acetylcholine and histamine are chosen which give responses of similar size and are applied alternately. Atropine is then added to the bath, the acetylcholine response rapidly declines in size but the histamine response remains unchanged. This suggests that histamine is not combining with the same receptor as acetylcholine (and atropine). It might be possible to find an antagonist that is selective for the histamine receptor. In fact many such antihistamines are known of which mepyramine (p. 179) may be used as an example. If a suitable concentration of mepyramine is now added to the Ringer solution in the organ bath, the histamine response is reduced rapidly without any effect on the acetylcholine re-

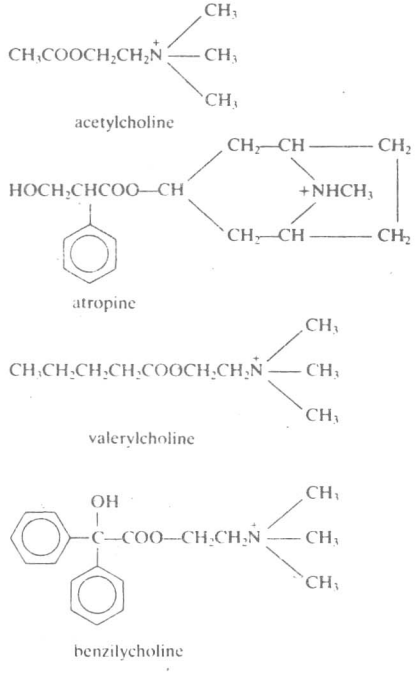


Fig. 1.7.

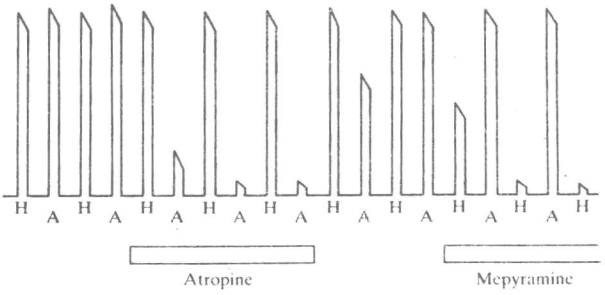


Fig. 1.8. The ileum was made to contract by addition of alternate doses of acetylcholine (A) and histamine (H). Altropine selectively antagonizes the acetylcholine effects and mepyramine the histamine effects.

sponse. The experiment shows how in the use of selective antagonists we have a very satisfactory and discriminating criterion for distinguishing between types of receptors and hence between groups of drugs.

So far we have considered the action of acetylcholine on one tissue, the smooth muscle of the ileum. We need now to ask the question, whether the characteristics of acetylcholine action on other tissues are the same. Acetylcholine also acts on skeletal muscle, being in addition the chemical mediator at motor nerve terminals. We can conveniently study structure–activity relationships by using the rectus abdominis muscle of the frog, which responds to acetylcholine by a contraction and can be set up in an isolated organ bath in a similar way to the ileum. When we look at the potency of the acetylcholine series on this tissue we find that propionylcholine is four times as active as acetylcholine, not one twentieth as active as it was on the ileum (Table 1.3), that butyrylcholine is also more active than acetylcholine, and that even valerylcholine retains about one third of the activity of acetylcholine. It is evident that acetylcholine is not the optimum structure on this tissue and that the **structure–activity relationships** are not the same as for the ileum. This is *prima facie* evidence that we are dealing with a different receptor and we would expect to find confirmation of this by the use of antagonists. Sure enough, atropine in concentrations that are strongly antagonistic in the ileum is totally without effect on the rectus. On the other hand, *d*-tubocurarine is a potent antagonist for acetylcholine on the rectus but is ineffective on the ileum. The existence of two distinct receptors for acetylcholine was first envisaged by Dale when he described two actions which he referred to as **muscarine-like** and **nicotine-like**. Dale was in fact comparing the two kinds of action we have just decribed. On smooth muscle, muscarine is very active and nicotine inactive, whereas the converse is true on skeletal muscle.

Table 1.3. Activity relative to acetylcholine. The relative potencies of a homologous series of choline esters in causing contractions of the guinea-pig ileum and the frog rectus abdominis muscle

	Ileum	Rectus abdominis
Formylcholine	25	100
Acetylcholine	100	100
Propionylcholine	5	400
Butyrylcholine	0.5	150
Valerylcholine	0	30

In fact, using the criteria that we have now established, i.e. structure–activity relationships in agonists and selective antagonists, we can show that muscarinic receptors for acetylcholine seem to be very similar whether they are in intestinal smooth muscle, the heart, blood vessels, salivary glands, or central nervous system. The nicotine type of receptor is found only in skeletal muscle, autonomic ganglia, and to a minor extent, in the central nervous system. The existence of a receptor

that is distributed in many tissues of course defines the basic spectrum of activity of the drug when administered systemically; it is no use expecting atropine to have a selective effect on the intestine, since it will also produce a drying of the mouth, increase in heart rate, and defective focusing of the eye, due to its action on the muscarinic receptors in these tissues.

Although receptors may have similar properties, as indicated by sensitivity to agonists and antagonists, this does not necessarily indicate that the effects produced by activating the receptor are the same.

For instance the action of acetylcholine on the muscarinic receptor in the intestinal muscle is to depolarize the muscle and to cause it to contract, whereas in the auricle it causes hyperpolarization and reduced contractility, and in the salivary gland hyperpolarization and secretion. Therefore, it is evident that the muscarinic receptor is in some way coupled to a variety of *effector* mechanisms that initiate the physiological responses that are produced.

Drug receptors

At this point we have arrived at the very useful concept that there is a specific tissue component, which we have called the drug receptor, with which antagonists and agonists interact. We assume that something about the nature of this interaction determines the character and specificity of drug action. How can we obtain more direct information about this interaction? A method that has been very successful is to measure directly the binding of a radioactively labelled drug to a tissue or some partly purified fraction of the tissue. Initially let us use the muscarinic receptor for our example. We will take a homogenate of rat brain in which the cells have been broken into fragments and suspended in a saline medium and add to it a radiolabelled antagonist, such as *N*-methyl atropine. Allow a few minutes for equilibrium to be established by the antagonist and then separate the cell fragments from the supernatant by centrifugation and measure the amount of radioactivity in the two fractions. If this experiment is repeated with differing amounts of antagonist a binding curve can be generated as is shown in Fig. 1.9. This has a sigmoid shape just like the dose–response curves we have considered previously and a maximum amount of binding is achieved, which in this case was 1.2 nmol of antagonist per gram of protein in the homogenate. We now need a simple theory of binding of a drug to a receptor in order to characterize the binding further.

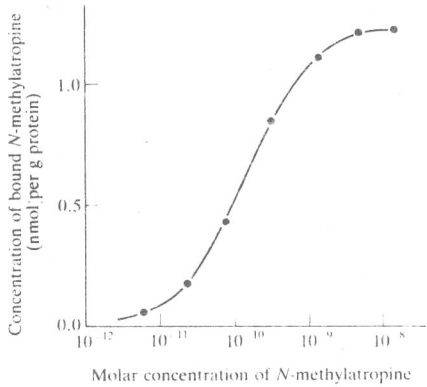


Fig. 1.9. The binding of tritium-labelled *N*-methylatropine to a cell membrane preparation from rat brain. The maximum amount bound corresponds to the amount of receptor present and is 1.2 nmol/g of protein.

The simplest model is that a molecular complex is formed between each receptor molecule and a single molecule of the antagonist.

$$D + R \underset{}{\overset{K}{\rightleftharpoons}} DR$$

The proportion of the receptor occupied by the drug (known as the occupancy and written as p) is given by the law of mass action as

$$p = \frac{D}{D + K}$$

where D is the concentration of free drug and K is the dissociation constant.

When $D = K$ then $p = 0.5$ and hence we can conclude that the drug concentration that produces half occupancy of the receptor gives the dissociation constant of the drug. The curve drawn in Fig. 1.9 fits the equation and

the dissociation constant is found in this case to be 1.4×10^{-10}M.

It is useful at this point to consider another useful way of handling binding data. If we rewrite the equation so as to include the concentration of receptors, we have

$$B = \frac{B_{max}\, D}{D + K}$$

where B is the amount of ligand bound and B_{max} the total concentration of binding sites.

This can be rearranged as follows

$$\frac{B}{D} = \frac{B_{max}}{K} - \frac{B}{K}$$

If we plot $B/_D$ against B (Fig. 1.10) we see that the intercept on the abscissa gives us the capacity B_{max} and the slope of the line gives $1/K$. This is called a *Scatchard* plot. It is the best method for determining the concentration of receptor and the dissociation constant of the ligand.

The direct binding method can also be used to prove that two drugs are interacting competitively into the same receptor site. In this case the fraction of receptor occupied by the first drug is given by

$$p = \frac{D/K_1}{1 + D/K_1 + A/K_2}$$

where K_1, K_2 are the dissociation constants of the two drugs, and A is the concentration of the second drug. In this method, from the displacement of the first drug (which is radioactively labelled) by the second drug A, we can find the dissociation constant K_2 of the second drug. But we can also find this directly by using radioactive A. If the two drugs are in simple competition for the receptor, the dissociation constant obtained by these two methods should be identical, as it is found to be.

Let us now apply the same kind of argument to antagonism in the smooth muscle experiments. We need make only two assumptions. First, that the level of contraction is dependent in some way on the proportion of receptors occupied by the agonist and second, that agonists and antagonists are in competition for the receptors.

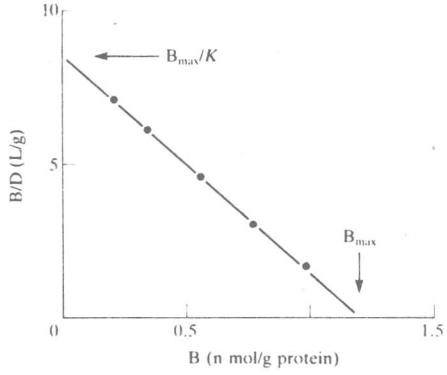


Fig. 1.10. Scatchard plot of radioligand binding.

The experimental procedure is to make a dose–response curve for the agonist in the absence and presence of a fixed concentration of antagonist and to find the doses that produce a particular level of response in the two cases. The ratio of these doses is called the **dose ratio** and is written d. The equation for antagonism now reduces to the very simple form

$$d - 1 = A/K$$

There should be a linear relationship between $d-1$ and the concentration of antagonist – this is indeed the case and this kind of curve (Fig. 1.11) is known as a **Schild plot**.

We can now compare the dissociation constants for the receptor determined in the pharmacological experiment with those determined by radioligand binding. They are identical!

Experiments of this type have been performed for a wide range of important pharmacological receptors. In most cases these quantitative relations hold, but in a few cases the relations are more complex.

The principles we have developed apply equally well to the competitive inhibition of enzyme action and of course enzymes can be important drug receptors.

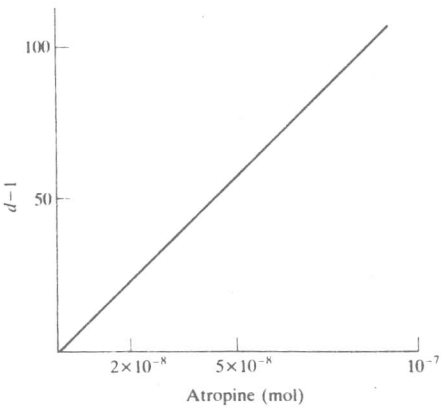


Fig. 1.11. Dose–ratio plot for the antagonism of acetylcholine by atropine.

What is the nature of drug receptors?

Drug receptors are macromolecular structures that are needed to provide a stable three-dimensional matrix with which the drug can interact and which can provide the necessary selectivity. The most well-studied drug receptors are soluble proteins, enzymes. In many cases the structures of enzymes have been extensively characterized by X-ray crystallography, by nuclear magnetic resonance spectroscopy and other methods both as an unoccupied receptor structure and with appropriate drugs in place. An example of an important drug receptor studied in this way is the enzyme dihydrofulate reductase, a small and simple protein of molecular weight 18,000 and containing amino acids in a single linear peptide. Figure 1.12 shows the three-dimensional structure of the complex of the enzyme with the drug trimethoprim.

Of the drug receptors discussed earlier in this chapter by far the best characterized is the nicotinic receptor. This has come about because the electric organs of certain fishes are a very rich and abundant source of the receptor, which has made it relatively easy to isolate and characterize. In the cell membranes of some electric organs nearly half the protein present is receptor protein. This receptor is complex and is compound of five protein subunits of 40–60 000 daltons molecular weight, only two of which are identical in structure. The two identical subunits bind the agonist, acetylcholine and one of the other subunits binds a modifying drug, histriconotoxin. The sequence of all the subunits has been determined by DNA sequencing techniques and they have been cloned. All four subunits have striking sequence homologies, which suggest that they have been derived by gene duplication from a single ancestral gene. The subunits can be separately purified and then recombined in an artificial membrane to

Fig. 1.12. The binding site on dihydrofolate reductase for Trimethoprim showing the amino acids in closest contact. (Kindly provided by B. Birdsall.)