REVIEW ARTICLE

THE RELATION BETWEEN CHEMICAL STRUCTURE AND BIOLOGICAL ACTIVITY*

Discussion of Possibilities, Pitfalls and Limitations

By J. M. van Rossum, Ph.D.

Department of Pharmacology, Catholic University of Nijmegen, the Netherlands

The number of new compounds being synthesised every year increases in an exponential way. Consequently investigations of the relation between chemical structure and biological activity similarly increases. Studies of this kind provide information about which moieties in the molecule are essential to obtain potent and specific drugs. Furthermore they may give a clue to the mechanism of the action of drugs.

Two major aspects of structure-activity studies raise questions. Firstly, how do alterations in the chemical structure alter the geometry of the molecule and its physical properties? Secondly, what does the fact of biological activity imply? Do different drugs produce the same kind of effect; do they have an identical mechanism of action?

In this review the value of studies on structure-activity, as well as some of the pitfalls and limitations of interpretation will be discussed. Work done on the pharmacology of the nervous system will be used to illustrate this discussion.

Action and Activity

Investigations of structure activity relationships will be abbreviated to SAR studies. This notation is vague because it is also frequently used for structure action relationship studies.

The action, or effect, of a drug is a qualitative phenomenon while its activity is a quantitative measure denoting how much effect is produced.

When the activity of a series of drugs is compared, as in SAR studies, it is essential that all members of the series cause the identical effect; and not only the same effect, but they also should have an identical mechanism of action. In many examples this is not so.

Different drugs may produce a similar effect by an entirely different mechanism of action. For instance, the blood pressure may be lowered by acetylcholine as a result of vasodilatation of arterioles, but also by histamine when large numbers of capillaries are themselves dilated. It is evident that when the observed effect is achieved by a complicated function, many drugs produce similar effects by acting in different ways while, on the other hand, a single drug may produce a divergency of actions. This is so with drugs acting in the intact animal and especially when they act on the central nervous system. The study of drugs on isolated organs makes things less complicated but it is not always possible to extrapolate *in vitro* findings to intact animals or to man.

* Based on a lecture given for the pharmacological day during the XXII International Congress of Physiological Sciences, Leiden, on September 18, 1962.

Therefore to reduce variables to a minimum the activity must be measured in unambiguous terms. Some knowledge of molecular pharmacology seems necessary for this purpose.

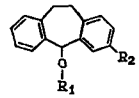
Interaction with Receptors

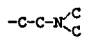
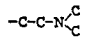
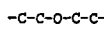
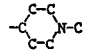
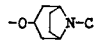
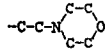
Most drugs are supposed to interact with receptive sites or receptors in the tissue. The molecular structure of the receptor and its physical and chemical properties largely determine what moieties in the drug are essential for affinity with the receptors. Other factors however also play a role.

Some receptors have strong structural requirements and therefore seem to be rather rigid, whereas others are very much less so. Thus the sites of activity of the volatile anaesthetics are hardly to be called receptors. The potency of these drugs depends largely on their being in the tissue fluid where chlartrate formation (Pauling, 1961) may occur, so that the electrical impedance of the tissue increases and conduction is impaired. Ferguson (1930) has calculated the activity, that is, the effective concentration, of volatile anaesthetics at their locus of action and was able to show that they differed little in potency. The geometric structure seems of little importance and there is not a great difference in potency between diastereoisomers and enantiomorphs.

TABLE I

SAR ON SPECIFIC AND NONSPECIFIC RECEPTORS, ACTIVITY ESTIMATED ON THE GUINEA-PIG ILEUM



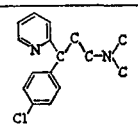
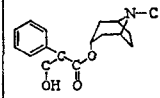
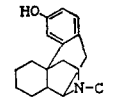
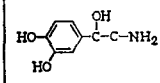
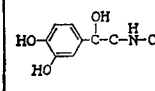
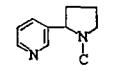
R_1	R_2	parasympatholytic (atropine) =10	histaminolytic (diphenhydramine) =10	spasmolytic (papaverine) =1
	-H	0.1–1	40 – 80	1 – 2
	-Cl	0.1–1	10 – 20	0.5–1
	-H	0.1–1	0.1 – 1	–
	-H	5 – 10	64 – 128	–
	-H	80–160	40 – 80	1 – 2
	-H	0.1–10	5 – 10	0.1–0.5

Other types of drug action involve a requirement for highly structural receptors as, for instance, parasympathetic receptors or histamine receptors. Examples are set out in Table I (Stelt, Harms and Nauta, 1961). From this Table it may be seen that changes in the molecular structure may cause the potency of the antihistamine or antiacetylcholine drug to increase sharply, whereas spasmolytic (papaverine-like) drugs acting on

the muscle directly, seem hardly to be influenced. The papaverine-like effects seem to depend on non-specific receptors. For drugs acting on inflexible receptors there may be a great difference in the activity of optical

TABLE II

DIFFERENCE IN AFFINITY OF OPTICAL ISOMERS

drug and (class)	formula	affinity ratio enantiomorphs
chlorpheniramine (antihistaminic)		12 (+)/(−)
hyoscyamine (p.sympatholytic)		100 (−)/(+)
levorphan (analgesic)		150 (−)/(+)
noradrenaline (α-sympathomim.)		3–4 (−)/(+)
isoprenaline (β-sympathomim.)		500 (−)/(+)
nicotine (ganglion stimulant)		equal

isomers. See Table II (Ariëns, 1962; Barlow, 1960; Beckett, 1959; Hanna, 1960; Kroneberg, 1955; Luduena, 1957, 1962; Schild, 1947).

BASIC STEPS IN DRUG ACTION

Since drugs are chemical substances an interaction of drug molecules with specific receptive molecules (receptors) in the biological tissue is an essential part in almost any form of drug action. For receptor occupation a sufficient number of drug molecules should be in the direct vicinity of the receptors. This environment has been called the biophase (Furchgott, 1955). The *first basic step* in drug action is therefore to discover how drug molecules reach the biophase and thus to build up an adequate concentration after administering the drug. It is obvious that this relation depends on the dose, the route of administration, on where the receptors are located in the tissue (on the exterior surface of the cell, or inside it, or in the brain) and on the biotransformation by enzymes.

In intact animals, and especially when drugs are acting in the brain, the relationship between the dose applied and the concentration in the biophase may be very complicated. Furthermore, the concentration in the

biophase is not constant but changes with time as a result of bio-inactivation, excretion and so on. Under standard conditions it is only possible to reach a steady state *in vivo* by infusion techniques.

Obviously the study of the action and activity of drugs on isolated tissue or enzymes is of advantage, since then the concentration in the biophase is more or less directly proportional to the dosage.

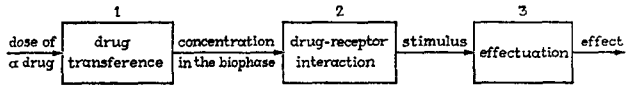


FIG. 1. Block scheme by which the sequence of events leading to the effect by application of a drug may be described. Block 1 represents the relationship between the dose applied and the concentration of the drug in the biophase. The influences of transport, metabolism, excretion etc. are collected in this block and headed under the term drug transference. The reaction of drug molecules with specific receptors under formation of the stimulus is presented in block 2, while the effectuation of the stimulus is given in block 3. From van Rossum (1958).

The *second step* is the drug receptor interaction proper (see Fig. 1). There is good evidence that the reaction between drug molecules and receptors is a bimolecular reaction (Ariëns and van Rossum, 1957b).

$$[R] + [A] \underset{k_2}{\overset{k_1}{\rightleftharpoons}} [RA] \qquad (1)$$

where [R] is the concentration of free receptors, [RA] the concentration of occupied receptors and [A] the concentration of drug A. There is no estimate of the total amount of receptors in a tissue so that we can calculate only the fraction of receptors ([RA]/r) occupied by the drug:

$$[RA]/r = 1/(1 + K_A/[A]) \quad .. \quad .. \quad .. \quad (2)$$

where r is the total number of receptors and K_A is the dissociation constant of drug A. The affinity of a drug A is defined as the reciprocal of the dissociation constant. The logarithm of the affinity equals the negative logarithm of K_A.

As a consequence of receptor occupation by an agonistic drug a stimulus is generated (Ariëns, van Rossum and Koopman, 1960; van Rossum, 1958; Stephenson, 1956).

$$S_A/S_m = \alpha.[RA]/r \quad .. \quad .. \quad .. \quad (3)$$

where S_A/S_m is the numerical value for the stimulus generated by a certain dose of drug A, while S_m is the maximum possible stimulus to be obtained by the particular tissue. The proportionality constant, α, has been called the "*intrinsic activity*" (Ariëns, 1954; Ariëns, van Rossum and Simonis, 1956a,b).

The intrinsic activity determines whether the drug can generate a stimulus and therefore whether a drug can be "active" or not. The affinity and the intrinsic activity are determined by the molecular properties of both receptor and drug. In structure activity studies these parameters must therefore be determined in the most direct way.

The *third basic step* is the effectuation of the stimulus, that is, the relationship between stimulus and ultimate effect. This relation is not dependent on the drug but determined by the tissue or animal. It is obvious that in isolated organs this relation may be relatively simple and often linear. But, in the intact animal and especially with drugs acting in the brain, the stimulus-effect relationship may be extremely complicated. Furthermore in intact animals there may be a feedback circuit which stabilises the system and counteracts disturbances caused by the drug. This is especially so for endocrine functions and the blood pressure.

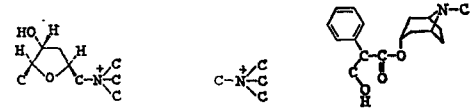
In the study of drug action, and therefore also in SAR studies, the effect of a single dose of a drug or the dose which causes a certain effect, is often measured. In certain cases dose-response curves are determined, here, the effects of various doses of the same drug are estimated. The potency of drugs may be calculated from the single dose-technique or from dose-response curves. It should be emphasised that the activity so measured depends on all three basic steps of the scheme in Fig. 1. Therefore it may not be concluded that the potent drugs in a series have the best fit on the receptor unless drug transference to the biophase is identical and the effectuation of the stimulus is a linear function. By using enantiomorphs we often eliminate differences in drug transference but not in effectuation. In such a situation the single dose-technique is not conclusive, since the activity so measured is a function of both intrinsic activity and affinity. These are the limitations of SAR studies and may be the source of large errors when conclusions about the configuration of the receptors are drawn from SAR studies.

Intrinsic Activity

It must be stressed that for SAR studies both the affinity *and* the intrinsic activity should be measured. The intrinsic activity is a measure for the agonistic potencies of a drug. Thus true agonists have a high intrinsic activity whereas competitive antagonists have a low intrinsic activity. This is analogous to substrates and competitive inhibitors in enzymology; the former have a real k_3 value, the latter a low or zero k_3 value (van Rossum and Hurkmans, 1962).

TABLE III

MOIETIES FOR AFFINITY AND INTRINSIC ACTIVITY



	muscarine	natural	tetramethylammonium	atropine
affinity	high		low	high
intrinsic activity	high		high	low

Different moieties may be responsible for intrinsic activity and affinity. So the conformation is important for affinity in muscarine while the

positively charged ammonium group is important for the intrinsic activity (see Table III). For sympathomimetic drugs the amino-group is important for intrinsic activity on α-receptors but the phenolic OH-groups are essential for intrinsic activity on β-receptors (Ariëns, 1960, 1962; Ariëns and Simonis, 1960).

Stereoisomers often differ in activity (see Table II). This in general is attributed to a difference in affinity, that is, the one substance fits well while its enantiomorphs fit badly with the receptor. However for some drugs the enantiomorphs have similar affinity but different intrinsic activity. This is so for isoprenaline on α-sympathetic receptors (Ariëns, 1962; Luduena, 1962).

The Measurement of Affinity and Intrinsic Activity

We have emphasised that it is important to determine both affinity and intrinsic activity instead of merely a potency or activity. How is such a differentiation made? It cannot be done by the estimation of equiactive doses of drugs; it can be done only by making dose-response curves and, even then, there are pitfalls and restrictions. Dose-response curves can

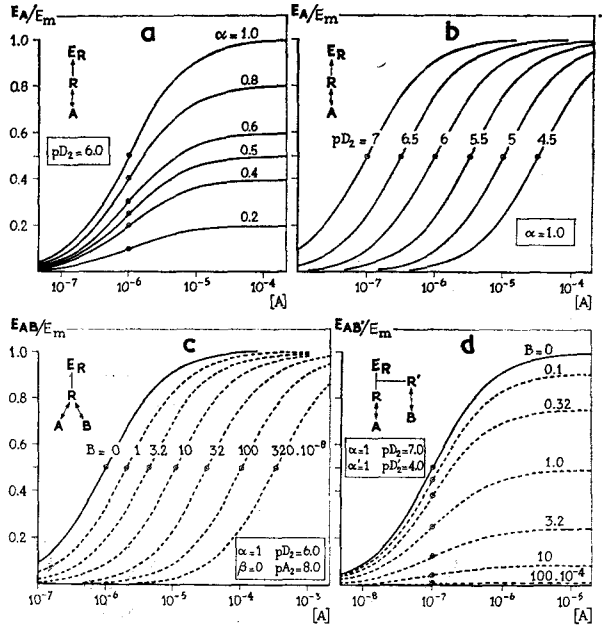


FIG. 2. Theoretical dose-response curves. (a) Dose-response curves calculated for drugs with equal affinity ($pD_2 = 6$) and different intrinsic activities. The maximum height of the curve is directly correlated with the intrinsic activity. (b) Dose-response curves calculated for drugs with equal intrinsic activity ($\alpha = 1$) but different affinities. The position of the curve on the dose axis correlates with the affinity. (c) Dose-response curves for an agonist in the presence of a competitive antagonist. The dose-response curves remain of identical shape and are merely shifted to higher doses along the dose axis. (d) Dose-response curves for an agonist in the presence of different concentrations of a non-competitive antagonist. Dose-response curves are reduced in height whereas the position on the dose axis remains unchanged.

only be made for agonists since antagonists are inactive on their own. The maximum height of the dose-response curves is a measure of the intrinsic activity while the position of the curve on the dose axis is a measure of the affinity (see Fig. 2a, b). The logarithm of the affinity ($-\log K_A$) can be calculated as the negative logarithm of the dose which causes 50 per cent of the maximum possible effect. This parameter is denoted the pD_2 value (Ariëns and van Rossum, 1957a).

$$pD_2 = -\log[A]_{50}$$

where $[A]_{50}$ is the concentration of A which causes an effect of 50 per cent (ED50). The intrinsic activity can be calculated only by reference to a standard agonist (S)

$$\alpha = E_{Am}/E_{Sm}$$

where α is the intrinsic activity, E_{Am} is the maximum effect of drug A and E_{Sm} is the maximum effect of the standard drug. It should be noted that the α and pD_2 values so obtained are true values for intrinsic activity and affinity only of an agonist under ideal conditions, that is, when there is a linear relationship of the factors involved in drug transference and

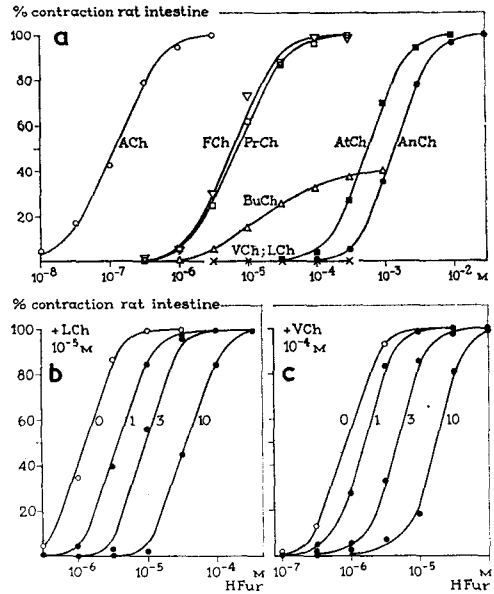


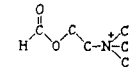
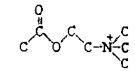
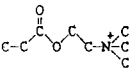
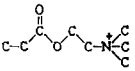
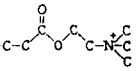
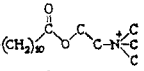
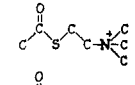
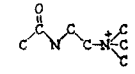
FIG. 3. Dose-response curves for acetylcholine analogues as studied on the isolated rat intestine. (a) Changes in the molecular structure of acetylcholine reflects in changes in affinity and intrinsic activity. Acetylcholine is the most potent drug of this series, whereas acetylthiocholine has a very low affinity but still high intrinsic activity. Butyrylcholine has an intermediate intrinsic activity, valerylcholine and laurylcholine are inactive of their own, Ariëns and Simonis (in the press); Koopman (1960); van Rossum and Hurkmans (1962). (b) Dose-response curves of an agonist in the presence of laurylcholine. The dose-response curves are shifted to higher concentrations along the dose axis indicating a competitive antagonism. (c) Similarly valerylcholine behaves as a competitive antagonist.

similarly with those involved in effectuation (Fig. 1). If, for instance, the stimulus-effect relationship is not linear, the pD_2 may be found too high and the intrinsic activity too low. For details see the literature (Ariëns and others, 1960; Nickerson, 1956; van Rossum, 1958; van Rossum and Ariëns, 1962; Stephenson, 1956).

In Fig. 3, dose-response curves are given for a homologous series of acetylcholine analogues as parasympathetic drugs. In the parent compound, acetylcholine, the methyl group of the acid part is replaced by an alkyl group of increasing length. It may be seen that the affinity decreases gradually but that there is also a gradual decrease in intrinsic activity. The higher homologues are themselves inactive. Formylcholine and acetylthiocholine have a low affinity but high intrinsic activity.

TABLE IV

SAR OF ACETYLCHOLINE DERIVATIVES

formula	drug	α	pD_2	pA_2	affinity ratio
	FCh	1	5.2	–	1.6
	ACh	1	7.0	–	100
	PrCh	1	5.3	–	2
	BuCh	0.5	5.1	–	1.2
	VCh	0	–	4.7	0.5
	LCh	0	–	5.4	2.5
	AtCh	1	3.3	–	0.02
	AnCh	1	3.0	–	0.01

Ariëns and Simonis, 1963.
Koopman, 1960.
van Rossum and Hurkmans, 1962.

Since antagonists are inactive by themselves they cannot be studied when given alone. Competitive antagonists lack the intrinsic activity but have affinity for the same receptors as the corresponding agonists. They can therefore be investigated in combination with a specific agonist. A parameter comparable to the pD_2 value of an agonist is the pA_2, as introduced by Schild (1947, 1949) for a competitive antagonist. Under ideal circumstances the pA_2 value is equal to the negative logarithm of the affinity of the competitive antagonist. A competitive antagonist causes

a parallel shift of the dose-response curve of a pure agonist (see Fig. 2c). The degree of shifting is a measure of the affinity:

$$pA_2 = -\log[B]_x + \log(x - 1)$$

where $[B]_X$ is the dose of the antagonist which causes a shift of a factor x (Schild, 1957). In Fig. 3b the experimental dose-response curves for a parasympathomimetic and an apparently inactive drug (antagonist) are given. The intrinsic activities and affinities for a series of acetylcholine derivatives are given in Table IV.

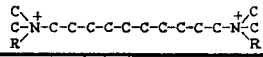
Antagonists are not necessarily competitive with the agonist. Some antagonists do not react with the receptors for the agonist but interact with other, although interdependent, receptors. They lack a structural feature that makes the drug fit well on the specific receptors through which the effect of the agonist is brought about. Thus they have their point of action beyond the specific receptors (Ariëns, Simonis and de Groot, 1955; Ariëns, van Rossum and Simonis, 1956b,c) (see block 3 in Fig. 1). These non-competitive antagonists do not cause a shift in the dose-response curves of the agonist but cause a diminution of its maximum height (see Fig. 2d). This property is used to calculate the affinity of the non-competitive drug. A parameter similar to the pD_2 value of an agonist has been denoted the pD_2' value (Ariëns and van Rossum, 1957).

$$pD_2' = -\log [B]_{50}$$

where $[B]_{50}$ is the dose of the non-competitive drug that causes a 50 per cent reduction of the dose-response curve of the agonist. Under ideal conditions the pD_2' value equals the logarithm of the affinity of the non-competitive antagonist. Since the non-competitive drug counteracts the

TABLE V

SAR OF DECAMETHONIUM DERIVATIVES, EXPERIMENTS ON THE ISOLATED RECTUS ABDOMINIS OF THE FROG



R	specific receptors			non-comp. receptors	
	α	pD_2	pA_2	α′	pD_2'
-C	0.8	5.9	–	–	–
-C-C	0.3	5.2	–	–	–
-C-C-C	0	–	5.0	–	–
-C-C-C-C	0	–	5.9	-1	5.7
-C-C-C-C-C	0	–	6.2	-1	6.0
-C-C-C-C-C-C	0	–	5.9	-1	5.9
-C-C-C-C-C-C-C	–	–	–	-1	6.2
-C-C-C-C-C-C-C-C-C	–	–	–	-1	6.4

van Rossum and Ariëns, 1959a.

stimulus which is elicited by the agonists, its intrinsic activity has received a negative sign ($\alpha' = -1$). Intrinsic activities (α and α') and logarithm of affinities (pD_2, pA_2 and pD_2' values) for a number of decamethonium derivatives are given in Table V. A differentiation between agonists, competitive and non-competitive antagonists is essential in SAR studies although such drugs may *in vivo* produce a common effect.

AGONISTS AND ANTAGONISTS

Agonists are characterised by the parameters affinity and intrinsic activity but also by the receptors with which they interact (see Table VI). It is generally not possible to isolate receptors, so that in SAR studies the determination of drug parameters on isolated tissues or intact animals must suffice. It should however be ascertained that the various agonists react with the same receptors; this is not proved by the fact that the various agonists cause identical effects. One way to solve this problem is the use of a specific competitive antagonist in combination with the agonist. A specific competitive antagonist should cause the same degree of antagonism in a certain dose, irrespective of the agonist used. In isolated organs such a procedure can be followed but *in vivo* it may not be possible to do so.

TABLE VI

PARAMETERS FOR AGONISTS AND ANTAGONISTS

type of drug	receptor type	affinity	intr. act.
agonist	specific	pD_2	$\alpha=1$
comp. antagonist	specific	pA_2	$\alpha=0$
non-comp. antagonist	other (interdependent)	pD'_2	$\alpha'=-1$

In studying different histaminomimetic drugs, receptor identity could be proved by combining the agonists with a selective antihistamine drug (Schild, 1957). Irreversibly acting drugs may also be used to evaluate the receptor identity of agonists (van Rossum, 1958; van Rossum and Ariëns, 1962).

Different agonists may react with identical receptors but they also may reach an identical effect by reacting with different receptors. This means that different agonists may not have an identical mechanism of action. Drugs that cause an identical effect by reacting with different receptors are of a separate class and may be called functional synergists (Ariëns and others, 1956c; van Rossum, 1951; van Rossum and Ariëns, 1959b). Parasympathomimetic drugs and histaminomimetic drugs are functional synergists on the isolated guinea-pig intestine. A differentiation between functional synergists and competitive agonists can be obtained by using selective irreversibly-acting drugs. This implies that one kind of receptor can be blocked permanently whereas the other kind is not affected.

The picture of agonists may further be complicated by drugs acting indirectly by causing the release of an endogenous agonist. The dose-response curves of indirectly-acting drugs are not shifted to the right to the same degree by a selective competitive antagonist as those of directly acting drugs.

When inactive compounds are found in a series of agonists, this may mean either that the intrinsic activity is zero, or that the affinity is nil. If *in vitro* the affinity is very low, the compound does not show any effect. *In vivo*, however, this may not be so since it may have affinity with other receptors. If the intrinsic activity is zero, a drug is only apparently

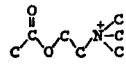
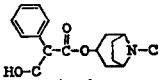
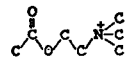
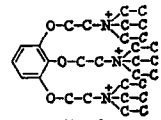
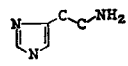
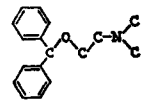
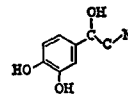
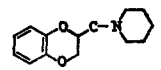
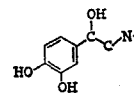
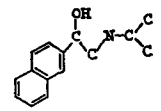
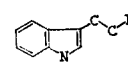
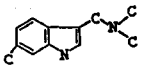
ineffective, since, if the drug still has affinity, it will interfere with receptor occupation by an agonist and thus show competition. In the SAR of the drugs shown in Fig. 3 and Table IV there is a change from agonist to competitive antagonist. This is a common situation when alterations are made in the molecular structure of an agonist.

An inactive drug which has no affinity to the specific receptors may, however, have affinity to non-specific (interdependent) receptors and so act as a non-competitive antagonist when combined with an agonist.

It must again be emphasised that "inactive" drugs are not necessarily inactive. They may be really inactive but, on the other hand, they may be competitive antagonists, non-competitive antagonists or functional agonists in addition. If therefore in a series of drugs, inactive members are encountered they should be studied as antagonists in combination with an appropriate agonist.

TABLE VII

SIZE OF AGONIST AND ANTAGONIST MOLECULES

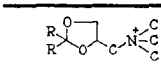
agonist	antagonist	receptor
 acetylcholine	 atropine	parasympathetic
 acetylcholine	 gallamine	myoneural
 histamine	 diphenhydramine	histaminic
 noradrenaline	 benzodioxane	α-sympathetic
 isoprenaline	 Nethalide	β-sympathetic
 5-hydroxytryptamine	 6-methylgramine	5-HT

Agonists are usually molecules of smaller size than the corresponding competitive antagonists (see Table VII). While the affinity of agonists is often not very high, competitive antagonists, which do not need moieties

for intrinsic activity, can be built for optimal affinity. There being competition, competitive antagonists interact with the same receptors as the corresponding agonist. Actually they do not react with identical receptors. The receptor area used by the antagonist is often larger. It is therefore dangerous to make pictures of the specific receptors for SAR studies of antagonists. A receptor supposed to be complementary to natural muscarine is quite different from the complementary part of hyoscyamine.

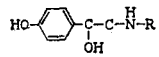
TABLE VIII

GRADUAL CHANGE FROM AGONIST TO ANTAGONIST VIA INTERMEDIATES WITH LOW AFFINITY

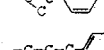
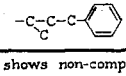


parasympathetic receptors (rat intestine)

R	α	pD_2	pA_2
H–	1	5.3	–
C–	1	4.3	–
C–C–	0.5	4.5	–
	0	–	4.4
C–C–C–	0	–	6.2
C–C–C–C–	0	–	6.9
	0	–	7.1



α-sympathetic receptors (rat v. deferens)

R	α	pD_2	pA_2
–H	1	4.6	
–C	1	4.1	
–C–C–C	0.5	3.0	
–C–C–C–C	–		< 2.0*
	0.2		2.5
	0	–	5.6
	0	–	5.5

* shows non-competitive antagonistic properties

Interpretation of in vivo *experiments*

Thus it is important to know whether drugs act as agonists or as antagonists. In isolated organs this is usually not a problem, but in intact animals, and especially with centrally-acting drugs, this may be extremely difficult to determine. For centrally-acting drugs we often do not know whether a stimulant drug really is an agonist or whether it is an antagonist. Strychnine for instance is considered a stimulant drug. It causes inhibition at inhibitory synapses (Eccles, 1961; Longo and Chiavarelli, 1962) but we do not know whether this inhibition is an agonistic action or whether there is competition with a transmitter so that its action would be antagonistic. A number of compounds of less

complicated structure have been synthesised (Chiavarelli and Setting, 1958) and screened for true strychnine-like action (Longo and Chiavarelli, 1962). These compounds have not been proved to be truly strychnine-like, while the inactive compounds do not yet seem to have been investigated for antagonistic properties. Studies along these lines are, however, promising.

A similar situation exists with morphine. Is the analgesic effect of morphine an agonistic or an antagonistic action? One might argue that presumably it is agonistic because *N*-allylnormorphine is a morphine antagonist. These drugs are mutual antagonists. We have seen that in most cases antagonists are obtained when larger substituents are introduced. It would then seem likely that morphine is an agonist.

It must be evident that, when larger substituents are introduced in drugs which are already antagonists, the derivatives remain antagonists. It is therefore not logical to anticipate that *N*-allylnoratropine would be an antagonist of atropine. Needless to say, a certain group in one type of drug may produce a totally different effect in another class of drugs.

The fact that competitive drugs are generally of a larger size than the agonists may explain why different series of antagonists, although being competitive with the same agonist, have entirely different requirements for optimal affinity. The different kinds may have affinity with different regions adjacent to the receptor for the agonist. This also may explain why in agonistic molecules small alterations, decreasing the intrinsic activity, also cause a decrease in affinity, while the introduction of aromatic (flat) ring systems increases the affinity again (see Table VIII). The weakest drugs of such a series may show non-competitive antagonistic properties, that is, they may react with other receptors and so interfere with the action of the agonist. The more potent agonists or antagonists may also have non-competitive affinity as well, but in low doses they are specific agonists or antagonists and at high doses they show additional effects. In fact most drugs have a multiple mode of action but they are selective when the ratio between affinity for the desired and undesired receptors is high. Drugs which have a very high affinity for certain kinds of receptors are, in general, specific. This is so since they fulfil the requirements for the particular kind of receptors and thus have little chance of being optimal for another kind, unless the two are strongly interrelated (see also Table I).

Tissue and Species Differences for Drug Parameters

The drug parameters, affinity and intrinsic activity, are determined by the molecular configuration and physical properties of the drug as well as those of the receptor. Specific receptors, as, for instance, parasympathetic receptors, may differ for different tissues of the same animal and similar tissues of different species. This may reflect in tissue and species differences with respect to affinity and intrinsic activity.

Van Rossum and Ariëns (1959c) have reported on a homologous series of dioxolanes. These parasympathetic drugs were studied on the isolated rat intestine, the isolated frog heart and on the blood pressure of the

anaesthetised cat. With the introduction of heavier substituents there was a decrease in the intrinsic activity so that the lower members which were agonists, gradually changed into competitive antagonists. On the frog heart the transition from agonist to antagonist took place earlier than on the intestine while on the blood pressure of the cat the drugs remained agonists still longer. Thus certain members of the series were pure agonists on one tissue but pure antagonists on another.

Similar results have been obtained for neuromuscular blocking agents on the frog rectus abdominis on the one hand and the chicken muscle on the other hand. Some of these drugs are depolarisers on the chicken but pure competitors on the frog rectus (van Rossum and Ariëns, 1959a).

The transition compounds are of special interest in SAR studies. When the action of a homologous series is studied the variable effect of the transition compounds can be understood. However, when such an intermediate drug is investigated alone, it is difficult to classify because of the variable response in different tissues of the same animal and similar tissues of different species. A nice example is the action of pilocarpine. Pilocarpine is considered to be parasympathomimetic but owing to its relatively large molecular size the intrinsic activity can hardly be as great as that of acetylcholine. In fact, on the intestine, pilocarpine was found to have an intrinsic activity of about 80 per cent of that of acetylcholine (van Rossum 1960b). Since on the heart the intrinsic activity is found to be lower, pilocarpine behaves largely like atropine. When studied on the pupil of the eye extremely variable results may be obtained. Depending on the amount of endogenous acetylcholine released from nerve endings, which causes the pupil to constrict, to a certain degree pilocarpine may either cause a miosis or a mydriasis. On a pupil largely constricted it acts like atropine, while on some animals pilocarpine may even be inactive.

Molecular Conformation and Pharmacological Activity

Flexible drug molecules may acquire many possible conformations. Depending on the flexibility of the receptor, sometimes a few, sometimes more, drug conformations may give optimal affinity. Rigid drug molecules which have the correct configuration may have a perfect fit. However in such an example small alterations may reflect great differences in affinity.

There is good evidence that, for instance, parasympathetic and sympathetic receptors are rigidly built, whereas the cholinergic receptors in autonomic ganglia seem to be rather flexible (see below). For rigid receptors there will be a strict SAR and there will then exist a great difference in activity of optimal isomers. If the one isomer fits in an optimal way its enantiomorph does not fit or hardly fits. Table II gives a few examples.

If the enantiomorphs of a drug are both antagonists, a difference in total activity is due to only a difference in affinity. But, for agonists, the enantiomorphs may differ in affinity as well as in intrinsic activity. Since the geometry of the molecule is important for receptor occupation an activity difference for enantiomorphs is for a great part attributed to

affinity, while a difference in intrinsic activity may be an additional factor. It is unlikely that the enantiomorphs have identical affinity but differ only in intrinsic activity.

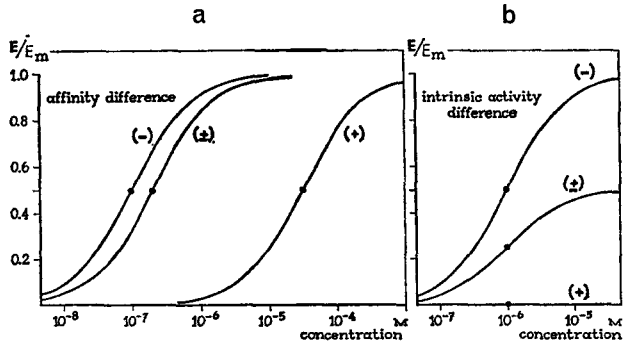


FIG. 4. Theoretical dose-response curves for enantiomorphs and the racemate. (a) The (−)-isomer is considered to have an affinity 300 times higher than the (+)-isomer, while both are considered to have identical intrinsic activity. In such a case the racemate seems to have an affinity of half that of the (−)-isomer. In the racemate the (+)-isomer does not contribute to the effect because of the high potency of the (−)-isomer. (b) The (−)-isomer is considered to have a high intrinsic activity ($\alpha = 1$), whereas the (+)-isomer is considered to have a low intrinsic activity ($\alpha = 0$). Both isomers have equal affinity. The racemate then seems to have an intrinsic activity in between, because equal amount of receptors are occupied by an agonist (−) and by competitive antagonist (+). This remains so over the dose-response curve.

In Fig. 4 dose-response curves are given for (+)-, (−)- and the racemate of theoretical compounds. In the left figure it is assumed that the affinity of the (−)-compound is 300 times higher than that of the (+)-isomer while the intrinsic activities are identical. The racemate is then practically half as potent as the (−)-isomer. In the right figure it is assumed that both isomers have identical affinity but that the (−)-compound has a high intrinsic activity and therefore is a pure agonist, while the (+)-isomer has a low intrinsic activity and therefore is inactive by nature. Actually it acts as competitive antagonist of its enantiomorph. The racemate then seems to have an intrinsic activity in between, which is due to a competition between the enantiomorphs. Since both isomers have equal affinity, at every dose level of the racemate they occupy equal amounts of receptors. That implies that the (−)-isomer can occupy only maximally 50 per cent of all receptors and therefore produce only 50 per cent effect. Obviously a drug with an intrinsic activity equal to 0·5 can also maximally produce 50 per cent effect but in this example all receptors are occupied while the contribution to the effect per occupied receptor is only half of that of a pure agonist.

There are many examples of agonists which differ only in affinity. For instance (+)- and (−)-muscarine and (+) and (−)-methacholine on parasympathetic receptors (Ellenbroek and van Rossum, 1960; Waser, 1958, 1961) and (+)- and (−)-adrenaline on α-sympathetic receptors (Ariëns, 1960, 1962; Hanna, 1960) (see Fig. 5). There are only a few

examples of enantiomorphs with equal affinity but with a difference in intrinsic activity. This is so for (−)- and (+)-isoprenaline (Ariëns, 1962; Luduena, 1962). The racemic mixture apparently has an intermediate intrinsic activity (Ariëns, 1962) (see Fig. 5c).

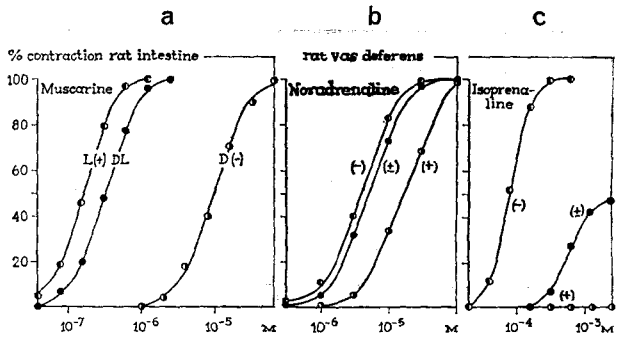


FIG. 5. Dose-response curves of enantiomorphs and their racemate. (a) Enantiomorphs of muscarine as parasympathomimetics on the isolated rat intestine. The L(+)-isomer is twice as potent as the racemate and about 300 times more potent than the D(−)-isomer. The difference in potency is attributed to a difference in affinity. The isomers have equal intrinsic activity (Ellenbroek and van Rossum, 1960). (b) The enantiomorphs of noradrenaline as α-sympathomimetics of the vas deferens of the rat. The (−)-isomer is slightly more potent than the racemate and about 4 times more potent than the (+)-isomer. The different potencies are attributed to differences in affinity. The intrinsic activities are identical. (c) The enantiomorphs of isoprenaline as α-sympathomimetic drugs. The (−)-isomer is an agonist whereas the (+)-isomer is inactive of its own. The racemate shows an intermediate intrinsic activity. The differences in potency are largely attributed to differences in intrinsic activity (Ariëns, 1962).

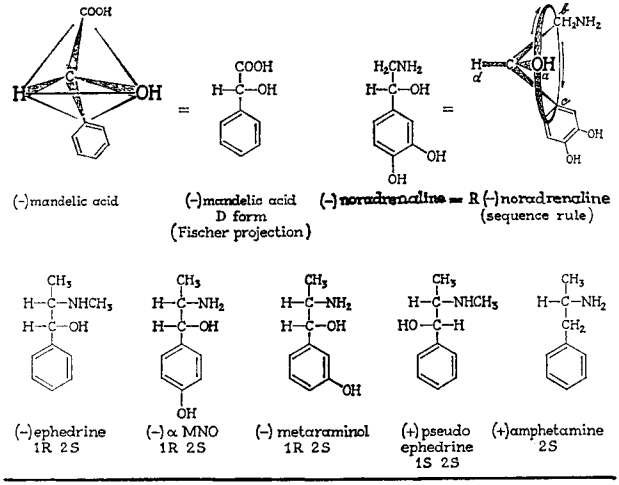
SAR studies of series of enantiomorphs may provide valuable information on the configuration of the receptor since the drugs with optimal fit are more or less complementary with the receptor. Then obviously the absolute configuration of the various active isomers should be known. The optical rotation does not give information about the absolute configuration.

An unambiguous notation of absolute configuration should be used in publications otherwise an enormous amount of confusion will ensue. The Fisher notation D and L seems accurate only for α-amino-acids and α-hydroxycarbonic acids which contain only one asymmetric centre. The sequence rule as introduced by Cahn and Ingold (1951) and Cahn, Ingold and Prelog (1956), meets a need. Knowledge of the sequence rule is a necessity for studying the relationship between geometrical structure and biological activity. In Table IX the absolute configuration of a number of sympathetic drugs is given according to the sequence rule, while furthermore the relationship between the Fisher projection and sequence rule is briefly outlined. The notation *R* (rectus) and *S* (sinister) is found as follows. The four substituents around the asymmetric centre are arranged according to their weight (sequence a, b, c, d) for which rules have been given (Cahn and Ingold, 1951; Cahn, Ingold and Prelog, 1956). The group of lowest weight (d) is placed in the

axis of a steering wheel. The three other groups complete the steering wheel. The wheel is then rotated in the direction from a → b → c. If such a rotation is to the right the configuration is *R*, and when it is to the

TABLE IX

NOTATION OF ABSOLUTE CONFIGURATION



Pratesi and others, 1958, 1959.
Dijk and Moed, 1961.
Dirkx, 1962.

left the configuration is denoted as *S*. For details see Cahn and others (1951, 1956). The relationship between absolute configuration and affinity and intrinsic activity of muscarinic drugs is given in Table X.

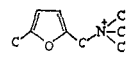
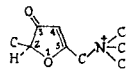
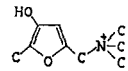
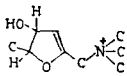
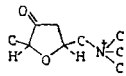
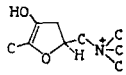
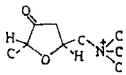
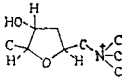
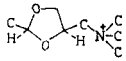
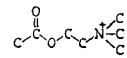
It has been proved by Pratesi, La Manna, Campiglio and Ghislandi (1958, 1959) that natural noradrenaline (laevorotatory) and natural adrenaline have the *R*-configuration. For closely related compounds such as (—)-β-hydroxytyramine and (—)-noradrenaline an optical rotation of the same sign may indicate identical configuration although it is not a proof. For metaraminol (*m*-hydroxynorephedrine) which is also laevorotatory not even a suggestion may be made that the corresponding OH group would have the same configuration as natural adrenaline because two asymmetric carbon atoms are involved (see Table IX).

Rotation dispersion determinations may help in ascertaining the absolute configurations of similar drugs (Dirkx, 1962; Lyle, 1960). It could be proved with rotation dispersion that (—) α-methylnoroxedrine, α-MNO (Table IX) has identical configuration with (—)-ephedrine (Dirkx, 1962). This conclusion had been reached by van Dijk and Moed (1959, 1961) by using chemical methods. By using rotation dispersion techniques Dirkx (personal communication) has recently established the absolute configuration of (—)-metaraminol and (—)-cobefrine (3,4-dihydroxynorephedrine) as 1*R*: 2*S*.

When alterations are made in drug molecules changes in both steric factors and in other physical properties are involved. Since the chemical and physical properties of enantiomorphs are largely identical, the only differences are steric features. In general, it may be assumed that the factors of drug distribution are identical for enantiomorphs so that in most instances both isomers acquire the same concentration in the biophase. This implies that if we cannot avoid the biophase relations, as in intact animals, it is useful to work with stereoisomers. Obviously this is not so if active transport or asymmetric metabolism is concerned.

TABLE X

CONFIGURATION AND ACTIVITY OF MUSCARINICS

drug	formula	configuration	form studied	α	pD_2	relative potency
methyl-furmethide		planar		1	7.2	160
dehydro-muscarone (cis)		2S	(±)	1	7.6	400
tautomer		planar		1		
dehydro-muscarine (trans)		2S : 3R	(±)	1		100
muscarone		2S : 5S	(−)	1	7.8	700
tautomer		5S		1		
allo-muscaron		2R : 5S	(±)	1		170
muscarine		2S : 3R : 5S	(+)	1	7.1	120
methyl-dilvasène		2S : 5S	racemates	1	7.3	200
ACh		—	—	1	7.0	100

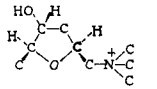
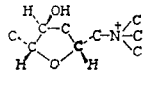
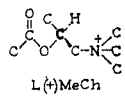
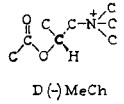
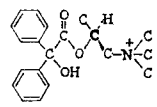
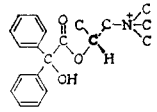
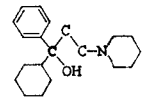
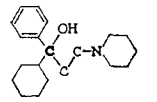
Gyermek and Unna, 1960.
van Rossum and Ariëns, 1959b.
Waser, 1958; 1961.

Laarhoven, Nivard and Havinga (1961) have recently studied stilboestrol derivatives in which effects other than steric effects were minimised. They found that steric hindrance to coplanarity of dimethoxystilbenes is one essential factor for oestrogenic activity. Angles of twist larger than 60° sharply increase activity.

It has been pointed out by Pfeiffer (1956) that optical isomers with high activity show a great difference in activity of the isomers. This rule seems to hold for all possible kinds of drugs. Evidently this rule can hold only if the asymmetric centre in the molecule is in a key position with respect to affinity or intrinsic activity (see Table XI). From this Table it may be seen that for the optical isomers of the potent parasympathomimetics muscarine and methacholine there is a great difference in affinity. Both the active muscarine and methacholine have the same configuration (Beckett, 1962; Ellenbroek and van Rossum, 1960). The corresponding asymmetric carbon atom is in a key position.

TABLE XI

KEY POSITION AND ASYMMETRIC CENTRE

enantiomorphs		affinity ratio
		$L/D=300$
L(+)muscarine(natural)	D(-)muscarine	
		$L/D=300$
L(+)MeCh	D(-)MeCh	
		$L/D=1$
L(+)BIP	D(-)BIP	
		$(-)/(+)=150$

Ellenbroek and van Rossum, 1960.
Ellenbroek, 1963.
Long and others, 1956.

Now if, instead of the acetyl ester from *S*- and *R*- β-methylcholine, an ester is made with benzylic acid, potent atropine-like drugs are obtained (Ellenbroek, 1963). The high affinity of these esters is due to the phenyl rings which may form van der Waals' binding with an appropriate part in, or near, the receptor. Now there is practically no difference between the enantiomorphs. The asymmetric carbon atom is not in a key position (see Table XI). If however the asymmetric centre is located on the negative site of the molecule again a great difference in affinity of the enantiomorphs is found (Long, Luduena, Tullar and Lands, 1956). The rule of Pfeiffer is valid when the receptors are inflexible molecules and the asymmetric centre is in a key position. Not only for enantiomorphs does it hold that the difference between them is great when the potency is high. It also holds in a more general way for the specificity

of the drug. A drug with an optimal fit on one kind of receptor will, in general, fit badly on other receptors. This is because requirements for the one differ from those of another. Drugs with a low affinity and having a poor fit on specific receptors, but having similar affinity for various sorts of receptors, are therefore not selective. It will be found that small alterations in potent drugs may reflect great variations in drug parameters whereas alterations in the molecule of weak drugs hardly elicit a change.

The considerations which have been discussed will now be applied to SAR studies of some selected classes of drugs acting on the peripheral and central nervous system.

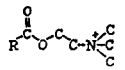
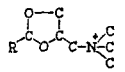
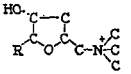
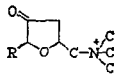
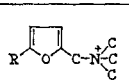
PARASYMPATHOMIMETIC AND ANTI-ACETYLCHOLINE DRUGS

Parasympathomimetic drugs are related to acetylcholine in their molecular structure. They have affinity to specific receptors in cholinergic synapses and in addition a real intrinsic activity. They therefore are often called cholinomimetics. Those which have only affinity are sometimes called cholinolytics.

In the peripheral nervous system, three types of cholinergic receptors can be distinguished. They are the postganglionic parasympathetic, the ganglionic and myoneural receptors.

TABLE XII

SAR OF POTENT PARASYMPATHOMIMETICS

formula	R	drug	α	pD_2	affinity ratio
	H–	FCh	1	5.2	1.6
	C–	ACh	1	7.0	100
	H–	HF	1	5.3	1
	C–	MeF	1	7.3	100
	H–	(±) trans HMu	1	4.8	1
	C–	(±) Mu	1	6.8	100
	H–	(±)H-muscaron	1	5	2.5
	C–	(±)-muscaron	1	7.6	100
	H–	HFur	1	5.9	6
	C–	MeFur	1	7.2	100

Koopman, 1960.
van Rossum and Ariëns, 1959a.
van Rossum and Hurkmans, 1962.

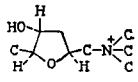
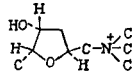
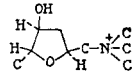
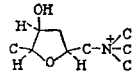
The parasympathetic receptors seem inflexible and consequently there is a strict structure-activity relationship. The agonists on parasympathetic receptors are very similar in molecular configuration (see Table XII). There is a great difference in potency of enantiomorphs.

It is evident that conclusions about the structure of the parasympathetic receptors may not be drawn from SAR studies in which only the overall

activities of the structural analogues are compared. A frequently used test for parasympathomimetics (muscarinics) is reduction of the amplitude of the frog heart set up according to Straub. Obviously the heart can be stopped beating in a number of ways. Using as a basis the dose which causes a 25 per cent reduction of the heart amplitude it may be concluded that the four stereoisomers of muscarine differ greatly in parasympathomimetic activity (see Table XIII) (Waser, 1961). The epi-isomer is the weakest muscarinic drug. However, when both affinity and intrinsic activity are determined, it becomes evident that the epi-isomer is largely an atropine-like substance (van Rossum, 1960a). So the activity determined in the "usual" way may lead to incorrect conclusions. From this series of compounds it may be concluded that minor changes in the molecular configuration may cause a change from agonist to antagonist.

TABLE XIII

ACTIVITY OF DIASTEREOISOMERS OF (±)-MUSCARINE AS ESTIMATED ON THE ISOLATED FROG HEART

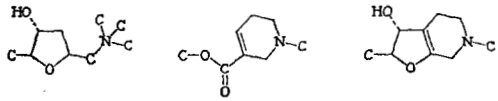
formula and configuration		drug	relative activity	α	pD_2	pA_2
	2S:3R:5S	(±)-Mu	100	1	6.4	–
	2R:3R:5S	(±)-epi-allo Mu	0.27	1	4.5	–
	2R:3S:5S	(±)-allo-Mu	0.21	0.4	–	3.7
	2S:3S:5S	(±)-epi-Mu	0.015	0.1	–	3.8

van Rossum, 1960a.
Waser, 1958.

When determining both affinity and intrinsic activity, a distinction can be obtained about which moieties are responsible for affinity, that is, for the binding capacity with the receptor, and which moieties are responsible for the intrinsic activity, that is, for the capacity to act as a mimetic. The quaternary nitrogen atom is essential for the mimetic action of muscarinic drugs while the conformation or configuration is important for affinity. Introduction of heavier substituents on the nitrogen atom causes a gradual change from parasympathomimetic to anti-acetylcholine properties. As a rule, the affinity decreases simultaneously with the intrinsic activity, unless planar ring systems are introduced which increase affinity by combining with adjacent regions near the receptor proper. Compare the structure of atropine and other potent antiparasympathomimetics with the mimetics or the antagonist obtained by gradually introducing heavier substituents in a mimetic drug.

Muscarinic Sites

The potent muscarinic drugs have a rather rigid structure (see Tables X, XII). The quaternary nitrogen atoms of this molecule, however, may still acquire many different conformations. In arecoline, which is also a potent muscarinic drug, the nitrogen atom is in a fixed position in the ring but in this molecule the negative site or the ester configuration may

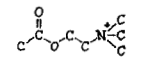
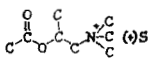
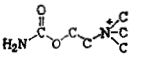
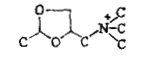
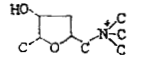
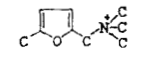
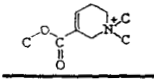


muscarine arecoline rigid drug

acquire different conformations. It seems tentative to speculate that in muscarine the nitrogen atom may be in a configuration similar to that in arecoline while in arecoline the ester grouping may in a similar way be held as the negative side of muscarine. Combining these two configurations, an extremely rigid molecule may be built, which should then be a potent muscarinic drug. This awaits further confirmation. The conclusion must be that unless the conformation of a drug when it is on the receptor is known, it is still difficult to draw conclusions about the receptor being complementary to the drug.

TABLE XIV

SAR OF POTENT PARASYMPATHOMIMETICS

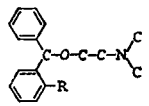
agonist quaternary	rat intestine		agonist tertiary	rat intestine	
	α	pD_2		α	pD_2
	1	7.0	nor-ACh	1.0	4.5
 (+)S	1	6.9	nor-MeCh	0.9	3.9
	1	7.0	nor-CarbCh		
	1	7.3	nor-MeFMe$_3$	1.0	4.2
	1	7.1	nor-muscarine	0.9	3.8
	1	7.2	nor-MeFurMe$_3$	0.8	4.3
	0.9	4.8	arecoline	1.0	6.3

The nitrogen atom in arecoline is tertiary. If this nitrogen atom is made quaternary the intrinsic activity as well as the affinity decreases. This in contrast to acetylcholine and muscarine (see Table XIV). When

these compounds are made tertiary there is a tremendous decrease in affinity and intrinsic activity. That the nitrogen atom in arecoline is in the ring is not the reason, since very closely related analogues of arecoline have been made (Jaramillo, 1962). For these analogues the quaternary compound was always more potent than the tertiary. The abnormal behaviour of arecoline has not yet been explained. Atropine-like drugs of other antimuscarinic drugs do not need moieties for intrinsic activity. It is therefore irrelevant whether the nitrogen is tertiary or quaternary (this is only important as far as biophase relations are concerned). Optimal affinity seems to be achieved in different ways.

TABLE XV

2-ALKYLDIPHENHYDRAMINES, ACTIVITY ESTIMATED ON THE ISOLATED GUINEA-PIG ILEUM



R	parasympatholytic activity (affinity)	histaminolytic activity (affinity)	ratio p/h
-H	1	1	1
-C	2.1	0.2	10
-C-C	4.2	0.1	42
-C-C-C	4.9	0.1	49
	6.5	0.1	65
-C-C-C-C	5.5	0.07	79
	16.0	0.1	160
	33.0	0.05	660
laevo	50.0	0.06	830
dextro	0.3	0.06	5

Harms, 1956.

Since anti-muscarinic drugs have no intrinsic activity the potency is a reasonable measure for affinity. In Table XV the SAR is given of a number of diphenhydramine derivatives (Harms, 1956). It must be concluded that substituents which prevent free rotation of the planar rings favour atropine-like properties and that there is a great difference in potency of optical isomers.

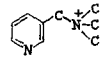
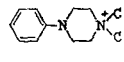
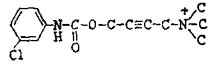
Ganglia

The cholinergic receptors in the ganglionic synapse seem to be more flexible. There is no such structure activity relationship (see Table XVI). In experiments where ganglionic stimulants were combined with a selective competitive antagonist, only 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP) and nicotine reacted with common receptors; the nicotine homologue 3-pyridylmethylyltrimethylammonium bromide, PMTM, reacted

at least with a partially different receptor (van Rossum, 1962). There was also no difference in activity of stereoisomers. Barlow (1960) has shown that *S*- and *R*-nicotine have identical activity. The cholinergic receptors in the autonomic ganglia differ to a great extent from those in the parasympathetic synapse. The potent and inflexible parasympathomimetics such as muscarine and dioxolanes are only weak ganglionic stimulants, whereas the flexible muscarinics acetylcholine and carbachol have strong ganglionic stimulating properties.

TABLE XVI

SAR OF GANGLIONIC STIMULANTS

formula	drug	guinea-pig intestine	
		α	pD_2
	nicotine	0.7	5.3
	PMTM	1.0	5.9
	DMPP	0.6	5.6
	McN-A343	0.9	4.8

van Rossum, 1962.

Skeletal-myoneural Junction

The cholinergic receptors in the myoneural junction seem to be similar to those in the ganglia but different from the parasympathetic receptors. In the myoneural junction the structure activity relationship among mimetics is not strict. But the nitrogen atom is important for the intrinsic activity in all cholinergic drugs. Introduction of heavier groups on the nitrogen atom of decamethonium, for instance, causes a gradual change from mimetic to lytic (see Table V). This implies a gradual change from a depolarising neuromuscular blocking agent into a non-depolarising competitive neuromuscular blocking agent. When curare-like compounds are studied with the head-drop technique, such a structure activity relation ship would not become evident. Using the head-drop technique in SAR studies may suggest that there is no relationship at all between structure and action. However, as previously pointed out, we can speak of a structure-activity relationship only when the drugs under study exert the same mechanism of action. When in decamethonium one methyl group is replaced by an alkyl chain of increasing length, there is a gradual change from mimetic to lytic, but, by further increasing the chain length, the non-depolarising drugs change their mechanism of action; for instance, the heptyl derivative is a pure non-competitive antagonist of acetylcholine (see Table V) (van Rossum and Ariëns, 1959). This compound shows affinity for different receptors and in such a way blocks neuromuscular

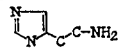
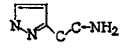
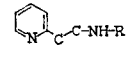
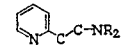
transmission. This clear structure-activity relationship did suggest immediately that a new clinical neuromuscular blocking agent, dioxahexadekanium bromide (G25178), might be of the non-competitive type. Indeed difficulties were encountered in classifying this compound, since apparently it did not belong to the depolarisers nor to the non-depolarising competitors. Actually it appeared to be a pure non-competitive antagonist of acetylcholine and therefore is a nice example of a new type of neuromuscular blocking agent (van Rossum, Ariëns and Linssen, 1958).

Histaminomimetics and Histaminolytics

Drugs which together with histamine have affinity and intrinsic activity on histamine receptors may be called histaminomimetics, whereas those which only have affinity to histamine-receptors may be called histaminolytics. Histaminolytics are competitive antagonists of histamine. Therefore many antihistamine drugs, are histaminolytics, but this is not necessarily so for all of them. At present it is not known which moieties in the molecule are essential for intrinsic activity on histamine receptors although the free amino-groups seem to have some significance. When in 2-

TABLE XVII

Histamino-mimetics and -lytics

formula	drug	guinea-pig intestine		
		α	pD_2	pA_2
	histamine	1	6.5	–
	pyrazole-ethylamine	1	4.1	–
	R=H (pyrethamine)	1	5.4	–
	-C	1	5.3	–
	-C-C	0.9	4.3	–
	-C-C-C	0.1	–	4.0
	-C-C-C-C	0	–	4.2
	R =-C-C-C-C	0	–	6.0

Ariëns and Simonis, 1960.
Schild, 1957.

pyridylethylamine heavier substituents are introduced in the free amino-group the intrinsic activity gradually decreases so that finally histaminolytics or antihistamines are obtained (see Table XVII) (Ariëns and Simonis, 1960). Consequently the affinity also decreases so that some of the intermediate compounds have such a low affinity for the specific receptors that they behave as non-competitive antagonists. The affinity of the lytics so obtained can be increased by introduction of larger groups of planar ring systems, so apparently, for the lytics, regions adjacent to the receptor for histamine are important for the antihistamines. This suggests that the moieties in histaminolytics, which resemble histamine, are of minor

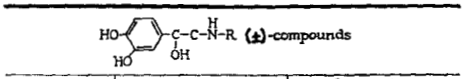
significance for receptor occupation and that additional moieties mainly determine the affinity. It is then conceivable that the antihistamine drugs and for instance, the atropine-like drugs, have more in common than their respective mimetics.

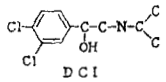
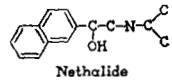
SYMPATHOMIMETIC DRUGS

According to Ahlquist (1959) a distinction can be made in sympathomimetic drugs between those predominantly acting on α-receptors and those acting on β-receptors. There exist α-sympathomimetics which have both affinity for α-receptors and an α-intrinsic activity, and "α-sympatholytics" which are competitive antagonists of α-mimetics. Furthermore there are β-sympathomimetics which have both β-intrinsic activity

TABLE XVIII

HOMOLOGOUS SERIES OF SYMPATHETIC DRUGS ON α- AND β-RECEPTORS



R	α-receptors (vas deferens)			β-receptors (tracheal muscle)		
	α	pD_2	pA_2	α	pD_2	pA_2
–H	1	5.4	–	1	5.8	–
–C	1	5.7	–	1	6.7	–
	0.4	3.4	–	1	7.5	–
	–	–	< 2.5	1	7.6	–
	0	–	5.5	1	8.1	–
	0	–	5.2	1	8.2	–
 DCI				 Nethalide		
DCI	–	–	–	0	–	4.9
Nethalide	–	–	–	0	–	5.8

Ariëns, 1960.
Ariëns and Simonis, 1960.
Ariëns, Simonis, Waelen and Sonneville, 1963.

and affinity to β-receptors and "β-sympatholytics." The moieties for the two affinities and two intrinsic activities may be quite different. It should therefore be realised that in SAR studies of sympathomimetics, relative potencies may change and that one type of drug may gradually change into another. In this respect the α: β-affinity ratios and both intrinsic activities are of importance.

SAR studies of derivatives of (±)-noradrenaline are presented in Table XVIII. It may be noted that introduction of large substituents on the

nitrogen atom causes the α-intrinsic activity to decrease, whereas this operation does not influence the β-intrinsic activity. Furthermore the α-affinity decreases and with the introduction of planar rings it increases again. The β-affinity increases with the introduction of large groups. This implies that predominantly α-sympathomimetics (noradrenaline) change into β-sympathomimetics while the latter compounds in higher doses exert 'α-sympatholytic' properties. The nitrogen group is especially important for α-intrinsic activity whereas the catechol moiety is determinant for β-intrinsic activity. Therefore dichloroisoprenaline (DCI) and especially nethalide (Black and Stephenson, 1962) are β-sympatholytics (see Table XVIII).

In SAR studies it is advantageous to be able to study one particular type of action at a time. This may be achieved by investigating sympathomimetic drugs on the isolated vas deferens that responds only to α-sympathomimetics and isolated tracheal muscle of the calf which is suitable for β-sympathomimetics.

Both α- and β-sympathetic receptors are rather inflexible since there is a substantial difference in the potency of enantiomorphs. The OH group at the C(1) atom seems to be in a key position for both α- and β-receptors. For the β-receptors the difference in potency between the enantiomorphs is about 10–1000, whereas for the α-receptors between 3–10. This would imply that for the β-receptors the position of this OH-group is critical (Ariëns, 1962). It would be very important to resolve the optical isomers of the β-blocking drug, nethalide, to see whether the same configuration is essential for mimetic and blocking action. If the β-mimetic and the β-blocking combine with the same receptor, a great difference in affinity of enantiomorphs of nethalide would be anticipated. The unequivocal notation of configuration is essential for studies of configuration and sympathomimetic action. The use of *d* and *l* and D and L for rotation and configuration is highly confusing. The use of the sequence rule is pertinent.

It has been pointed out by Burn and Rand (1958), that sympathetic action can be brought about by drugs which do not react with the specific sympathetic receptors but which act by releasing endogenous sympathomimetics from storage sites. This is so with the closely related drugs amphetamine and tyramine. This further complicates the picture. It must be emphasised that in SAR studies these facts should be thoroughly realised.

PSYCHOMOTOR STIMULANT DRUGS

Typical psychomotor stimulant drugs are amphetamine, ephedrine and their derivatives. These compounds resemble the catecholamines, noradrenaline, and dopamine in the molecular structure. The relationship between structure and activity of many amphetamine derivatives has been examined by van der Schoot (1961) and van der Schoot and others (1962). Evidence from these experiments is presented in Table XIX. It is essential for psychomotor stimulant action that the drugs be stable and that they be sufficiently lipophylic to pass the blood brain barrier. The

TABLE XIX

SAR OF PSYCHMOTOR STIMULANT DRUGS MEASURED BY INCREASE OF LOCOMOTOR ACTIVITY IN MICE

drug	rel. potency	drug	rel. potency
 (dopamine)	0	 (phenylethylamine)	1-2
 (noradrenaline)	0	 (amphetamine)	100
	100	 (metamphetamine)	150-200
	35		60
	0		50
	0		15
	0		8
	0	 (didrex)	10
	15		6
 (ephedrine)	8	 (segontin)	0*
	50	 (phenmetrazine)	14
 (diethylpropion)	13	 (cocaïne)	25

* antagonist

van der Schoot, 1961.

potent drugs therefore have a substituent (e.g. methyl group) on the carbon atom next to the amino-group and they lack phenolic OH-groups.

Furthermore, considering the strict structure activity relationship this may imply that the various amphetamine-like compounds interact with common receptors. There are however some compounds which cause identical psychomotor stimulant actions to amphetamine but which have a completely different structure. One such drug is cocaine. Furthermore the *N*-benzyl derivatives of amphetamine, as for instance, the

compound (+)-*N*-benzyl-*N*-methylamphetamine (Didrex), do not show peripheral sympathomimetic actions like amphetamine but do show identical central effects. This may suggest that at least two classes of psychomotor stimulant drugs may exist as was anticipated by van der Schoot (1961).

It has been shown that psychomotor stimulant action is related to the function of catecholamines in the brain whereas the action of 5-HT is not involved (van Rossum, van der Schoot and Hurkmans, 1962; van der Schoot, 1961; van der Schoot and others, 1962; Smith and Dews, 1962). The receptors for catecholamines may be related either to the α-receptors of the peripheral nervous system or to the β-receptors. Since in most amphetamine-like drugs the β-hydroxy group is lacking, a resemblance to β-receptors is unlikely. This could further be established by using selective α- and "β-sympatholytics." The "α-sympatholytics" antagonise psychomotor stimulant action whereas the β-lytics do not influence this effect unless in toxic doses. Now it is likely that receptors for catecholamines in the brain are related to the α-receptors of the peripheral nervous system. We may make a scheme of adrenergic function in the central nervous

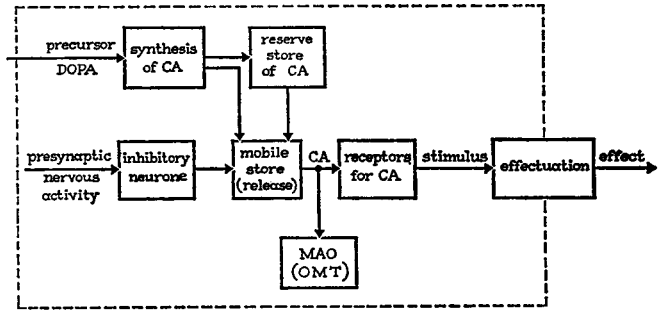


FIG. 6. Scheme of adrenergic function in the brain. The effect is proportional to the concentration of free catecholamines around the receptors. Under normal conditions catecholamines are released by "inhibitory" nervous activity, while catecholamines are continuously synthesised from precursors. Drugs may interfere with adrenergic function at the various blocks for instance by directly reacting with catecholamine receptors or by releasing catecholamines from stores. See text. In this scheme no distinction is made between dopamine and noradrenaline. Obviously in different parts of the brain dopamine and noradrenaline may have a completely different significance.

system (see Fig. 6). Under physiological conditions, catecholamines are synthesised from a precursor DOPA which can pass the blood brain barrier. The catecholamines synthesised are stored in either mobile or reserve stores. Under the influence of the physiological stimuli, catecholamines are released from the mobile stores while this store is refilled from the reserve. Once liberated, catecholamines may occupy specific receptors and elicit an increase in locomotor activity and other effects by a complicated route. The free catecholamines are also subject to metabolism by monoamine oxidase and by conjugation to 3-methoxy derivatives. Consideration of this scheme results in the following list of ten possible

mechanisms of psychomotor stimulant action: (i) drugs that act as precursors of catecholamines (CA); (ii) drugs that inhibit one or more steps in the synthesis of CA like α-methylDOPA; (iii) drugs that inhibit storage of CA; either in the mobile or reserve stores like reserpine; (iv) drugs that stimulate or inhibit the inhibitory neurones and so facilitate or inhibit release of CA from their stores; (v) drugs that cause a release of CA by acting on the stores; (vi) drugs that inhibit the release of CA by acting on the stores; (vii) drugs that inhibit monoamine oxidase or other mechanisms of destruction of CA; (viii) drugs that mimic CA by directly acting on specific receptors; (ix) drugs that antagonise CA by directly acting on specific receptors; (x) drugs that in some way facilitate or inhibit effectuation.

From the point of view of these possibilities, the drugs of immediate interest are those that have a *direct* sympathomimetic action (viii) and those that cause a rise in free CA and thus have an *indirect* sympathomimetic action (iv; v; vii).

Psychomotor stimulants may cause an increase in locomotion by directly reacting with catecholamine receptors or by causing a release of catecholamines from stores and thus causing the concentrations of catecholamines to rise. By pre-treatment with reserpine, catecholamine stores can be depleted so that drugs which act by releasing catecholamines would then become inactive. From experiments like these it could be shown that amphetamine has a direct mechanism of action, that is, amphetamine acts directly on catecholamine receptors, whereas cocaine has an indirect mechanism of action, that is, by releasing catecholamines from stores in the brain (van Rossum, van der Schoot and Hurkmans, 1962; van der Schoot, 1961). A further argument for the indirect action of cocaine is the following experiment: animals pre-treated with reserpine, so that catecholamine stores become depleted and cocaine becomes ineffective, are loaded with DOPA after which cocaine is again injected. DOPA is converted into catecholamines so that stores can be refilled when cocaine is active once more (van Rossum and others, 1962). The time between DOPA injection and cocaine restoration is less than an hour. This might suggest that dopamine instead of noradrenaline is involved in locomotion, since according to biochemical work (Hess, Connamacher, Ozaki and Udenfriend, 1961) the formation of dopamine from DOPA is a fast process, whereas the conversion of dopamine into noradrenaline is very slow. According to the biochemical findings, after one hour there cannot be enough noradrenaline in the brain. Furthermore, Eggels (1963) could not determine any significant amount of noradrenaline at the time that the cocaine effect was restored. At the moment experiments are being made to determine the dopamine concentration at the time of cocaine restoration. A further argument for the involvement of dopamine instead of noradrenaline is the failure to show tachyphylaxis with cocaine. When cocaine is administered at regular intervals, catecholamines are released from stores so that tachyphylaxis was anticipated. But the catecholamine stores can be refilled quickly with dopamine since there is a rapid synthesis from DOPA. The synthesis of noradrenaline from

dopamine, as shown by Hess and others (1961), is a slow process. For the peripheral nervous system it is more likely that noradrenaline is involved so that at that level tachyphylaxis may easily be observed after administration of indirect sympathomimetics.

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