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Review Article

A molecular basis for drug action*

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THE effect induced by a drug is the resultant of the interaction between the molecules of the drug and the molecules of which the biological object is composed. The higher the specificity required in the structure of the drug molecule, the more probable it becomes that the effect is based on an interaction of these molecules with certain specific molecules in the biological object. These specific molecules are called the receptors for the drug. They can be defined as those specific molecules, molecule complexes or parts of them in the biological object, with which the drug must interact in order to induce its effect. The term receptor goes back to Ehrlich (1913) who said: "Corpora non agunt nisi fixata". The notion of a specific receptive substance as a site of action for drugs such as nicotine and curare in the myoneural junction was introduced by Langley (1905). Since then the term receptor has become indispensable in reasonings on the basis of drug action. Often the model of key and lock is used for the drug-receptor interaction; reality is much more dynamic, however. Schueler (1960) defined the receptor as follows: "The drug-receptor is in general the pattern R of forces of diverse origin forming a part of some biological system and having roughly the same dimensions as a certain pattern M of forces presented by the drug molecule in such a way that between patterns M and R a relationship of complementarity for interaction exists."

Drug-receptor interaction must be seen as a mutual moulding of drug and receptor. There is mutual adaptation as far as shape and charge distribution is concerned. This adaptation plays an important role in the activation of drug and receptor and therefore is essential to drug action.

Drug-receptor interaction can have various consequences.

(*a*) The drug-receptor interaction mainly leads to changes in the charge distribution and shape of the drug molecule, in such a way that it is activated and becomes chemically more reactive, which results in chemical changes in the drug molecule. The drug is metabolised. The receptor is the "active site" on an enzyme.

(*b*) The drug-receptor interaction mainly leads to changes in the charge distribution on and in the shape of the receptor, so that as a result of this the receptor becomes activated and induces changes in the charge distribution and shape of the surrounding molecules, thus initiating the sequence of physico-chemical events leading to the effect.

(*c*) The drug-receptor interaction may take place without essential changes in the drug molecule or the receptor. No effect is then induced.

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This may be a binding to storage receptors, or, for example a binding to indifferent binding sites.

The receptors are characterised by the drugs with which they are able to interact and by the effect that can be induced on them. For drugs with low requirements for specificity in the chemical structure, sharply circumscribed receptors are less probable. In that case a more diffuse adsorption on some surface may take place. Especially then, properties such as the lipid solubility and surface activity of the compound are of importance.

Part-processes

Three groups of processes (van Rossum, 1958; Ariëns & Simonis 1961; Ariëns, 1962a) (see Fig. 1) are to be distinguished in the complicated process of drug action.

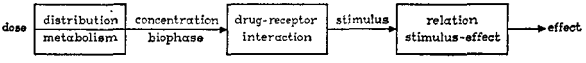


FIG 1.

RELATION BETWEEN DOSE AND CONCENTRATION

The processes which concern absorption, transport, biochemical changes, or excretion, of the drug (Brodie, 1956; Brodie & Hogben, 1957; Brodie, Gillette & la Du, 1958; Schanker, 1962; Williams, 1963). These determine the relation between the dose of the drug and its concentration in the immediate surroundings of the receptors, called the "biophase". (Furchgott, 1954, 1955.) If simple isolated organs are used as a test object, the concentration of the drug in the biophase may be supposed to be proportional to, or a simple function of, the concentration of the drug in the bath fluid.

THE DRUG-RECEPTOR INTERACTION

The processes concerned with the drug-receptor interaction and the induction of a stimulus by the drug. The interaction as such can be represented by a simple model based on the mass-action law or the Langmuir adsorption isotherm (Clark, 1937a, b). It describes the chance the molecules of the drug A have of interacting with the specific receptors R.

$$[A] + [R] \rightleftharpoons [RA]; k_2/k_1 = K_A \quad .. \quad .. \quad .. \quad (1)$$

The stimulus induced is supposedly proportional to the quantity of drug-receptor complex formed or present at a certain moment.

THE RELATION BETWEEN STIMULUS AND EFFECT

The processes which determine the relation between the stimulus induced and the effect obtained (Stephenson, 1956; van Rossum, 1958; Ariëns, 1962, 1964). There may be a graded response which means that the effect gradually increases with the stimulus and approaches asymptotically to some maximum value. Another possibility is the all-or-none

response in which case the effector cells respond only after the stimulus has reached a certain value, but then always with the same response per effector unit. The biological variance among the various effector units will have as a consequence a gradation in the response of the organ. In general, a certain value of the stimulus will always result in the same effect unless a change in the effector cells has taken place.

Depending on the parameter chosen as effect, a different number of steps in the sequence of events started by the drug-receptor interaction may be involved. Studying compounds which produce neuromuscular block, certain investigators use the head drop as the effect, others the decrease in contraction of the striated muscle to nervous stimulation; also the bio-electrical phenomena in the muscle fibre or at the endplate region can be measured as the effect, (Jenkinson, 1960) and so on. In all these examples the drug-receptor interaction and the stimulus induced are the same. The relation between stimulus and effect is usually complex, sometimes, however, for instance if simple isolated organs are used, it may be relatively simple.

With simple biological systems, such as isolated organs, the influence of drug metabolism can be neglected if stable compounds are used. The relationship between the concentration of the drug in the bath fluid and that in the biophase can then be assumed to be close to proportionality. For a certain type of organ and effect, the relation between stimulus and effect will be constant. The dose-response relation studied under these circumstances will mainly reflect the characteristics of drug-receptor interaction.

Affinity and intrinsic activity

As mentioned, the drug-receptor interaction results in a stimulus. This stimulus is supposedly proportional to the quantity of drug-receptor complex formed or present at a certain moment. Let us restrict ourselves first to the case when the effect is proportional to the stimulus and that the dose of the drug is large compared to the uptake capacity of the receptors. Application of the mass-action law shows that then, under equilibrium conditions, the effect E_A of the dose [A] as a fraction of the maximum effect E_m possible with the receptor-effector system concerned becomes:

E effector
|
R receptor
↕
A drug

$$E_A/E_m = \frac{\alpha}{1 + (K_A/[A])} \quad .. \quad .. \quad .. \quad (2)$$

K_A is the dissociation constant of the drug-receptor complex and the reciprocal of the affinity between drug and receptor. E_{Am} is the maximum effect obtainable with the drug A. With high doses of A the effect approaches the maximum value E_{Am}; then $E_{Am}/E_m = \alpha$.

If $E_A = \frac{1}{2} E_{Am}$, $k_2/k_1 = K_A = [A]$.

Besides the *affinity* between drug and receptor, the ability of the drug to interact with the receptors in an effective way, the *intrinsic activity* of the drug also is determinative for the effect (Ariëns, 1954; Ariëns, van Rossum & Simonis, 1956a, 1957; Ariëns, 1962b, 1964). The proportionality constant α in eqn 2 is a measure for the intrinsic activity of the drug.

To avoid dimensions for the intrinsic activity, it is expressed as the factor indicating the ratio between the maximum effect, E_{Am}, of the compound studied and the maximum effect possible with the receptor-effector system concerned, E_m. In practice, in a group of drugs with a common mechanism of action, the intrinsic activity of the compound producing the greatest maximum effect is taken as unity. For this reference compound, for example a compound B, the intrinsic activity has a value $E_{Bm}/E_m = 1$. The intrinsic activity of compound A then has a value E_{Am}/E_{Bm}. Once the physico-chemical processes at the basis of the intrinsic activity are known, dimensions can be applied and the term intrinsic activity can be avoided.

The effect is not necessarily proportional to the stimulus or to the fraction of receptors occupied. Where spare receptors exist, for instance, the intrinsic activity is not proportional to the maximum effect obtainable with the drug (van Rossum, 1958; Ariëns, van Rossum & Koopman, 1960; Ariëns, 1964). By definition the intrinsic activity is assumed to be always proportional to the maximum stimulus obtainable.

Active and "inactive" compounds

If log dose-response curves are studied for a homologous series of drugs in which a gradual change from active to inactive compounds takes place, the loss in activity may manifest itself in two different ways.

(i) The log dose-response curves may be shifted to higher and higher concentrations, so that finally extremely high doses of the drug are required, which implies that the compounds become practically inactive (Fig. 2).

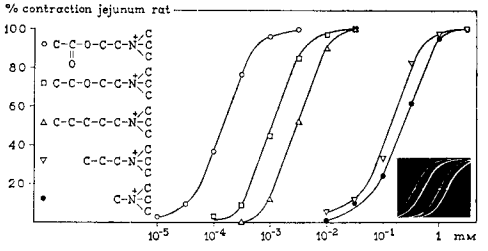


FIG. 2. Cumulative log concentration-response curves for a series of quaternary ammonium compounds. Note the decrease in activity as a result of changes in the chain manifests itself as an increase of the dose necessary to obtain the effect. Compare the experimental curves with the set of theoretical curves, inset, calculated from eqn 2.

(ii) A gradual decline in the maximal height and the slope of the log dose-response curves may take place which means that the compounds gradually become inactive within a reasonable dose range (Fig. 3).

The dose-response curves presented in the various figures are obtained with the cumulative dose-response technique described on a number of occasions (Ariëns & de Groot, 1954; Ariëns, Simonis & de Groot, 1955; van Rossum, 1958, 1963; van Rossum & van den Brink, 1963).

In terms of the model for drug-receptor interaction presented before (eqn 2), in case (i) the loss in activity may be ascribed to a loss in affinity, an increase in K_A; in case (ii) the loss in activity is possibly caused by a loss in intrinsic activity, a decrease in α. The structure-activity relation represented in Fig. 2 and 3 then indicates that the side-chain in acetylcholine mainly contributes to the affinity, while the cationic head appears to be of special importance for the intrinsic activity.

Competitive interaction

If the loss in activity, demonstrated in Fig. 3, is the result of a loss in intrinsic activity, the "inactive" compounds still have an affinity to the specific receptors and for this reason they may be expected to behave as

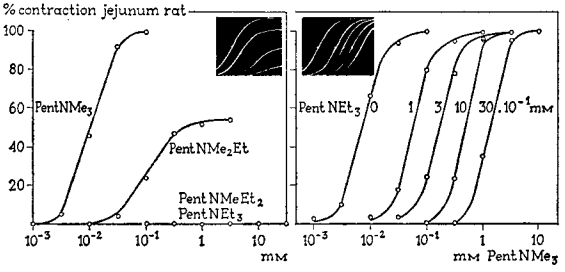
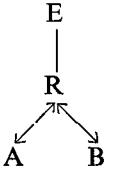


FIG. 3. (Left). Cumulative log concentration-response curves for a series of pentyl ammonium compounds. Note the decrease in activity as a result of the gradual ethylation on the ammonium group manifests itself as a decrease in the maximal effect and in the slope of the curves. Compare the experimental curves with the set of theoretical curves, inset, calculated from eqn 2.

FIG. 4 (Right). Cumulative log concentration-response curves for the agonistic compound pentylNMe$_3$ and the influence thereon of various concentrations of the "inactive" compound pentylNEt$_3$ (see Fig. 3). Note the parallel shift in the curves which indicates a competitive antagonism. Compare the experimental curves with the set of theoretical curves, inset, calculated from eqn 3.

competitive antagonists if combined with the active derivatives of the same series of compounds. Equations for the competitive interaction of two compounds can easily be derived (Ariëns, 1954; Ariëns & van Rossum, 1957; Ariëns, van Rossum & Simonis, 1957).

Eqn 3 gives the relations as derived from eqn 2 for the case when two drugs A and B, with intrinsic activities α and β, compete for common receptors.



$$E_{AB}/E_m = \frac{\alpha}{1 + [1 + ([B]/K_B)]\,(K_A/[A])} + \frac{\beta}{1 + [1 + ([A]/K_A)]\,(K_B/[B])} \quad .. \quad (3)$$

If $\alpha > 0$ and $\beta = 0$, B acts as a competitive antagonist of the agonist A. Eqn 3 then becomes identical to the well-known equation for competitive inhibition used in enzymology.

If the agonist A is combined with its competitive antagonist B, a parallel shift in the dose-response curve is expected if the curve is made in the presence of constant concentrations of the competitive antagonist. The antagonism is surmountable. An increase in the concentration of the agonist always results finally in a displacement of the antagonist from the receptors and therefore in a response. Fig. 4 gives the experimental results obtained with the combination of an active and an inactive derivative, from the series of compounds represented in Fig. 3.

In an homologous series of compounds in which the active derivatives gradually change to their competitive antagonists, transition compounds with an intermediate intrinsic activity, also called partial agonists, may be found. Take for instance the pentyl derivative bearing one ethyl group in Fig. 3 (Ariëns, 1954; Ariëns & Simonis, 1954; Stephenson, 1956; Ariëns, van Rossum & Simonis, 1957; Ariëns, 1964).

If in eqn 3, $\alpha = 1$ and $0 < \beta < 1$ is substituted, the compound B, a partial agonist, shows a dualism in action if combined with A; it acts as a synergist or antagonist depending on the value of [A]. An increase in the concentration of the partial agonist, B, if combined with constant concentrations of the agonist, A, will always result finally in the same response, independent of the concentration of A, because the partial

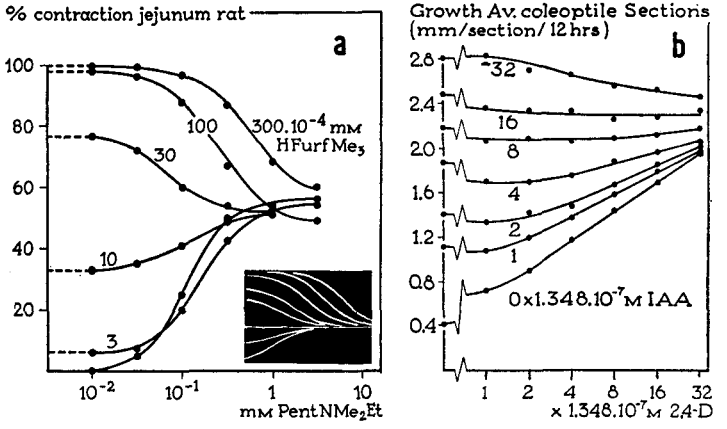


FIG. 5(a). Cumulative log concentration-response curves for the partial agonist $PentNMe_2Et$ and the influence thereon of various concentrations of the agonist furtrethonium ($HFurfMe_3$) (see Fig. 3). Note the dualistic character in the action of the partial agonist. Compare the experimental curves with the set of theoretical curves, inset, calculated from eqn 3.

FIG. 5(b). Log concentration-response curves for the partial agonist, 2,4-dichlorophenoxyacetic acid (2,4-D) and the influence thereon of various concentrations of the agonist indoleacetic acid (IAA). Both compounds act as auxins. Note the dualistic character in the action of the partial agonist. Compare the experimental curves with the set of theoretical curves, inset, calculated from eqn 3. (After McRae, Foster & Bonner, 1953).

agonist will finally always occupy all receptors. Fig. 5a gives the experimental results obtained with the combination of a partial agonist and a full agonist, from the series of compounds represented in Fig. 3. Fig. 5c

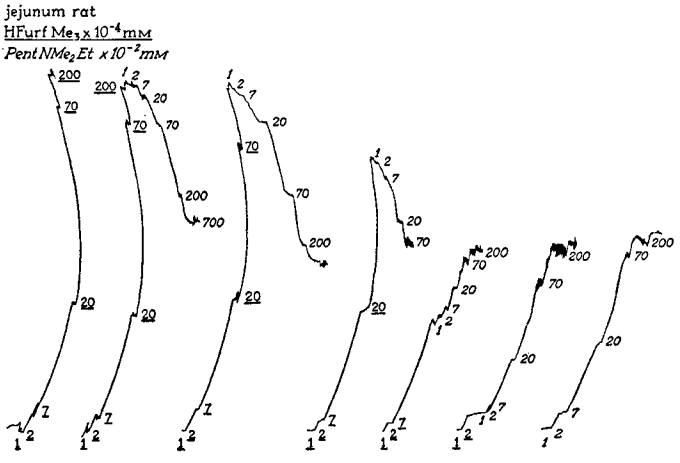


FIG. 5(c). A registrogram of cumulative dose-response curves for a combination of drugs of the type represented in Fig. 5(a). The underlined numbers concern the doses of the agonist furtrethonium ($HFurfMe_3$), the other numbers concern the doses of the partial agonist $PentNMe_2Et$. Note the dualistic character in the action of the partial agonist.

is the registrogram used for Fig. 5a. Fig. 5b gives an analogous set of curves for a combination of auxins. A gradual change from agonist to competitive antagonist via partia agonists has been described too for hormones such as polypeptides with an oxytocic action (Rudinger & Krejči, 1962). Theoretical curves which can easily be calculated from eqn 3 agree well with the experimental curves presented. If $\alpha = \beta > 0$, both A and B are agonists, and combination of these drugs will always result in a type of synergism, known as an additive action (Ariëns & Simonis, 1964).

The activity of agonistic drugs (stimulant drugs) is expressed by the intrinsic activity, which is proportional to the maximal effect obtainable with the drug and by the pD_2 value, the negative logarithm of that molar concentration of the drug that brings about an effect equal to 50% of the maximal effect obtainable with the drug, which represents the affinity (Ariëns & van Rossum, 1957). The activity of the competitive antagonists is expressed by pA_2 values according to Schild (1947); the intrinsic activity is zero (see Tables 1–3).

Molecular pharmacology and enzymology

In enzymology, a differentiation between substrates for an enzyme and specific inhibitors of the enzyme (competitive antagonists for the substrate) is well known. However, this differentiation is relative, because in a certain way each substrate will inhibit the breakdown of a related substrate

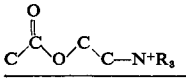
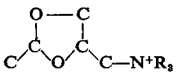
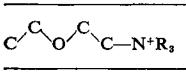
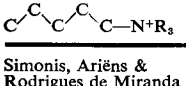
by the enzyme. Nevertheless it will not stop the enzyme action, because in protecting the related substrate it sacrifices itself. This means that the inhibitor does not necessarily act as an inhibitor as far as the formation of reaction products is concerned.

$$[E] + [S] \underset{k_2}{\overset{k_1}{\rightleftharpoons}} [ES] \xrightarrow{k_3} [E] + [S'] \qquad .. \quad .. \quad .. \quad (4)$$

A compound which has an affinity towards the active sites on the enzyme, but which is not broken down by the enzyme, ($k_3 = 0$), will act definitely as an inhibitor of the enzyme action (eqn 4). The term k_3 in enzymology is analogous to the intrinsic activity. The term $1/K_m = k_1/(k_2 + k_3)$ is analogous to the affinity.

In a series of related compounds a stepwise change from substrate to inhibitor—a gradual decrease in k_3—is possible. The intermediate

TABLE 1. INFLUENCE OF GRADUAL ETHYLATION OF PARASYMPATHOMIMETIC QUATERNARY AMMONIUM DERIVATIVES ON INTRINSIC ACTIVITY (i.a.) AND AFFINITY (pD_2 and pA_2 VALUES) (aff.) FOR THE MUSCARINIC ACTION TESTED ON THE JEJUNUM OF THE RAT

	Me_3		Me_2Et		$MeEt_2$		Et_3	
	i.a.	aff.	i.a.	aff.	i.a.	aff.	i.a.	aff.
	1	6·7±0·08	1	6·1±0·32	1	4·2±0·3	1	4·1±0·11
	1	7·1±0·29	1	6·4±0·32	0·3	4·0*	0	3·6*
	1	5·8±0·16	1	5·3±0·06	0	4·2±0·17	0	4·1±0·19
	1	5·4±0·23	0·65 ±0·18	4·3±0·33	0	4·2	0	4·6±0·30

Simonis, Ariëns & Rodrigues de Miranda (1964)

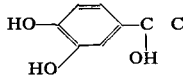
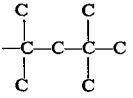
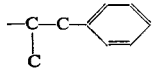
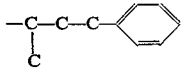
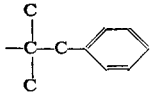
± Figures indicate standard deviations. * Van Rossum & Ariëns (1959).

TABLE 2. INTRINSIC ACTIVITIES AND AFFINITIES (pD_2 AND pA_2 VALUES) OF CHOLINE ESTERS, TESTED ON THE JEJUNUM OF THE RAT

Choline esters	Intrinsic activity		Affinity	
	mimetic	lytic	mimetic pD_2	lytic pA_2
Formyl-	1		5·2	
Acetyl-	1		7·6	
Propionyl-	0·9		5·0	
Isobutyryl-	0·4		4·1	
Butyryl-	0·3		3·8	
Capronyl-		0		4·0
Lauryl-		0		5·2
Diphenylacetyl-		0		6·7
Lachesine*		0		8·4

* Benzilic ester of monoethylcholine.

TABLE 3. INTRINSIC ACTIVITIES AND AFFINITIES (pD_2 AND pA_2 VALUES) OF NORADRENALINE DERIVATIVES, TESTED ON THE VAS DEFERENS OF THE RAT

	Intrinsic activity		Affinity	
	mimetic	lytic	mimetic pD_2	lytic pA_2
R = —H	1		5·4 (±0·2)	
—C	1		5·7 (±0·2)	
—C—C	0·94 (±0·05)		5·2 (±0·2)	
	0·4 (±0·1)		2·8 (±0·6)	
—C—C—C—C				<2·0
		0		3·5
		0		4·4 (±0·4)
		0		5·1 (±0·3)
		0		5·9 (±0·5)

The figures in brackets give the P_{95} for the mean value.

compounds can act as substrates as well as inhibitors; they show a dualism in action.

Fig. 6 gives log dose-response curves representing the quantity of acid formed per unit of time as a function of the concentration of the substrate for enzymatic hydrolysis of an homologous series of choline esters, including acetylthiocholine (AtCh), by acetylcholinesterase. AtCh has the highest rate of hydrolysis, therefore the largest value for k_3. Propionylcholine (PrCh) has an intermediate value, while butyrylcholine (BuCh) and laurylcholine (LCh) are hardly split at all. The substrate inhibition observed is not being considered. The Figs 7 and 8 give the experimental results for the enzymic hydrolysis of combinations of ACh and BuCh and of PrCh and AtCh. Compare the dose-response curves of Figs 6–8 with those in Figs 3–5 (van Rossum & Hurkmans, 1962).

Physico-chemical background of affinity

Besides those factors which determine the relation between the dose of the drug and its concentration in the direct vicinity of the receptors, the biophase, the dissociation constant of the drug-receptor complex in

particular, is determinative for the size of the effective dose. The affinity between drug and receptor, the reciprocal of the dissociation constant, in its turn depends on the reaction velocity constant, k_1, for the association and the velocity constant, k_2, for the dissociation. The value of k_1 is determined by the ease with which the drug molecule can detect the receptor. It will therefore depend especially on the long range electrostatic forces acting between drug and receptor. By these forces the drug

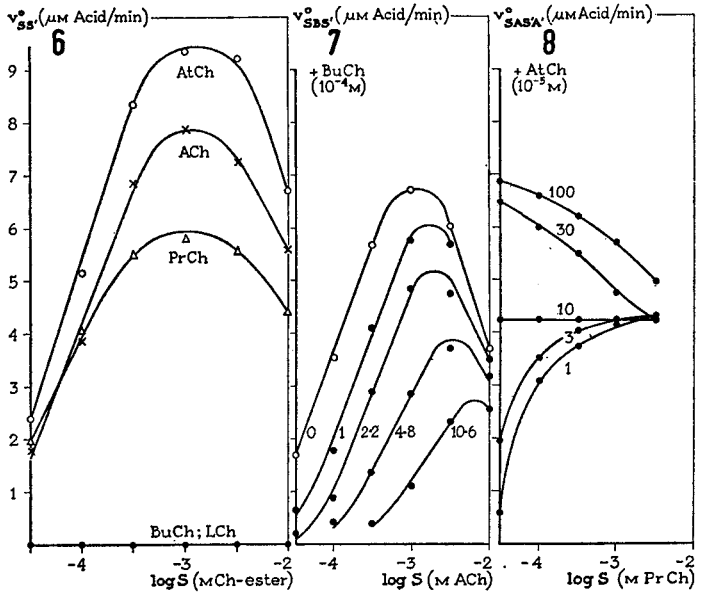


FIG. 6. Concentration-response curves for the enzymic hydrolysis (acid formation) of various choline esters. These curves were obtained by calculating the initial reaction velocity ($v^{\circ}_{SS'}$) for individual concentrations of the different substrates in a random order and under standard conditions. A 100 ml solution (0·1 M NaCl; $5{\cdot}10^{-5}$ M EDTA-Na) containing a certain concentration of a substrate was kept at pH 7·0 by an automatic titrator which regulated the flow of a 0·01 N NaOH solution. At zero time 1 ml of haemolysed human erythrocytes was added and the cumulative amount of alkali needed was recorded. The initial reaction velocity was calculated as the slope at the point of inflection of the obtained time-response curves. Note the difference in the maximal reaction velocity for the various substrates, acetylcholine (ACh), propionylcholine (PrCh) and acetylthiocholine (AtCh). Butyrylcholine (BuCh) and laurylcholine (LCh) are practically unhydrolysed (van Rossum & Hurkmans, 1962).

FIG. 7. Concentration-response curves for the enzymic hydrolysis (acid formation) of ACh and the influence thereon of butyrylcholine under similar conditions as those described in Fig. 6. A different batch of haemolysed erythrocytes was used. Note the parallel shift of the ascending limb of the dose-response curves of ACh, indicating a competitive inhibition (van Rossum & Hurkmans, 1962).

FIG. 8. Concentration-response curves for the enzymic hydrolysis (acid formation) of propionylcholine (PrCh) and the influence thereon of acetylthiocholine (AtCh) under similar conditions as those described in Fig. 6. Note with higher concentrations of PrCh the rate of acid formation approaches the value obtained with PrCh alone. At lower concentrations of AtCh, PrCh acts as a synergist, with higher concentrations of AtCh, PrCh acts as an antagonist. Compare with Fig. 5(a) (van Rossum & Hurkmans, 1962).

molecule is kept in the close vicinity of the receptor for some time, with the consequence that the chance that the molecule gets into the right position so that the short range van der Waals' forces between drug and receptor can come in action, is increased. As a result of the electrostatic attraction forces, the concentration of the drug molecules is increased in the border layer covering the surface bearing the receptors. As a consequence the ionic groups and other polar groups in drug molecule and receptor will be of special importance as far as k_1 is concerned. The reaction velocity constant for the dissociation, k_2, depends on the binding energy. Here, the van de Waals' forces which come into action once the drug molecule is adapted to the receptor, play an important role. The binding energy gained from electrostatic forces of the polar groups in drug and receptor, will remain relatively low as long as the interaction takes place in a medium of water rich in ions. The van der Waals' forces gained by the interaction of the lipophilic parts of the drug molecule and receptor, will make a large contribution to the binding and therefore to the affinity between drug and receptor.

Physico-chemical background of intrinsic activity

A wide variety of physico-chemical processes may be concerned with the induction of the stimulus. They vary with the type of stimulus induced and therefore with the type of effect studied. Some of the possibilities will be discussed in more detail.

EFFICACY OF SUBSTITUTES

The contribution to the stimulus and therefore to the effect may be constant for each individual interaction between a receptor and a molecule of a certain drug and vary for various drugs. The intrinsic activity then expresses this variation. Certain vitamin analogs may act in this way, after formation of a coenzyme analog and its binding to an apoenzyme to form a holoenzyme. The turn-over capacity of the enzyme analog will depend on the type of vitamin analog incorporated. The capacity may gradually change with its chemical structure and even decrease to zero. The compound then acts as an antivitamin. The intrinsic activity here expresses the gradation in the turn-over obtainable with the various vitamin analogs used as substitutes.

EFFECTIVE FRACTION OF COLLISIONS

It may be that only a fraction of the individual collisions between drug and receptor are effective, while the contribution to the stimulus is constant for each effective interaction. This fraction may vary for various drugs. There is an all-or-none response at receptor level. The intrinsic activity then represents the effective fraction of collisions (Ariëns, 1962, 1964).

The chance that the collision between a drug bearing a cationic group and the receptor having an anionic site will result in an ion-pair formation, will depend on the properties of the cationic group, e.g. a quaternary

ammonium group. Gradual ethylation on the onium group in acetylcholinomimetics may, because of steric hindrance, lead to an interference with ion-pair formation with as a consequence a decrease in intrinsic activity (Ariëns & de Groot, 1954; Ariëns, Simonis & de Groot, 1955; van Rossum, 1958; Ariëns, 1964) and a change to anticholinergic compounds (Figs 3, 4).

RATE OF COLLISIONS

Possibly the drug molecule contributes to the stimulus as long as it is on the receptor; as in the case of the vitamin analogs mentioned above, however, as postulated by Croxatto & Huidobro (1956) it may be that the molecule of the drug is effective only at the moment when its molecules are linked to the receptor. Durable linkage of the drug with the receptor does not then mean a lasting contribution to the effect. At the moment of linkage of the drug molecule to the receptor, free energy or an endogenous substance may be liberated, serving as, or contributing to a stimulus. Possibly the free energy must have a minimum indispensable quantal magnitude in order to be effective (Croxatto & Huidobro, 1956).

Recently, Paton (1961, 1964) combined the concept of the drug-receptor interaction based on the mass law and the postulate that only the rate at which the association between drug and receptor takes place determines the ability of a drug to contribute to the effect, or, in our terms, that the rate of association determines the intrinsic activity. As pointed out by Paton (1961, 1964), in equilibrium conditions, the rate of association is equal to the rate of dissociation of the drug-receptor complex, which is k_2 [RA]. In this model, called the rate theory, the equilibrium response obtained with a certain dose of drug A can be represented by:

$$E_A/E_m = \frac{k_2}{1 + k_2/k_1[A]} = \frac{k_2}{1 + K_A/[A]} \quad .. \quad .. \quad (5)$$

Compare this equation with eqn 2. The intrinsic activity, α, is substituted by k_2.

INDIRECTLY ACTING DRUGS

For indirectly acting drugs or liberators which act by virtue of endogenous substances, the rate of liberation and therefore the rate of drug-receptor interaction also may be determinative for the intrinsic activity. Examples of this type of action are the histamine liberators. It will be clear also that the rate of reloading of the system is then important.

In those cases in which the rate of drug-receptor interaction is essential, it is quite possible that only a fraction of the interactions are effective, which implies that the effect is determined by the product of the rate of association under equilibrium conditions, k_2 [RA], and a constant c_1 for the effective fraction of the associations. In these circumstances the equilibrium response obtained with a drug A can be represented by:

$$E_A/E_m = \frac{k_2c_1}{1 + k_2/k_1[A]} = \frac{k_2c_1}{1 + K_A/[A]} \quad .. \quad .. \quad (6)$$

where c_1 represents the effective fraction of the collisions. The intrinsic activity is determined by k_2c_1. Compare this equation with the eqns 2 and 5. The model postulated by Croxatto & Huidobro (1956) is an example. Not the rate of association, but the rate of *effective* associations—those which yield the minimum indispensable quantal magnitude of free energy—is supposed to be determinative for the intrinsic activity.

The fate of the constant α representing the intrinsic activity in eqn 2 will probably be the same as that of k_3 from the classical enzyme kinetics. It will be resolved in a variety of constants concerned with and depending on the sequence of chemical events which lead to the evocation of the stimulus (Ariëns, 1962). As long as equilibria or dose-response curves are studied, eqn 2 covers in a formal way the models represented by the eqns 5 and 6.

Fade and densensitisation

If the rate of association between drug and receptor is determinative for the intrinsic activity, a maximum will occur in the time-response curve immediately after application of the drug. This maximum is followed by a fade, a decrease, in the response to an equilibrium value. At the moment the drug is applied, all receptors are available for the interaction.

In the equilibrium state the number of receptors available is determined by the reaction velocity for the dissociation of the drug-receptor complex and/or possibly the rate of reloading in the case of liberators. The study of time-response curves may lead to a detection of those drug actions in which the rate of receptor occupation plays an essential role. Figs 9

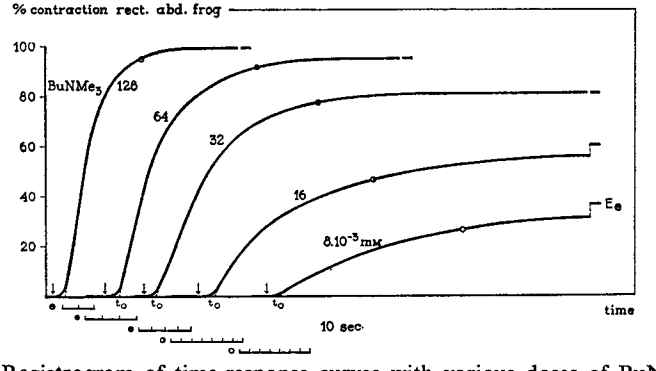


FIG. 9. Registrogram of time-response curves with various doses of $BuNMe_3$ on the isolated rectus abdominis muscle of the frog. The time scale varies for the various curves. E_e is the height of the contraction finally obtained with the dose concerned. ↓: addition of the drug. Note there is a gradual increase of the response with the time. No fade is observed in the response.

and 10 give such experiments for the action of acetylcholinomimetics on some effector systems. In these instances no indications are found of the maximum in the response or a fade phenomenon and therefore no indications for the rate of receptor occupation as determinant factor.

One of the problems in this respect is that it is often not the rate of receptor occupation but the rate of diffusion of the drug to the receptors that may be determinative for the time-response relationship.

Another implication of the rate theory is that after elimination of the drug from the bath fluid or biophase, the response becomes zero, although a fraction of the receptors may still be occupied by the drug. This involves a decrease in the response of the second and later doses of the drug, a desensitisation. There will be a cross desensitisation with respect to other drugs inducing their effect on the same receptors (Paton, 1961).

The desensitisation observed after application of high doses of acetylcholine to the isolated gut is not specific. Not only the sensitivity to acetylcholine but also to histamine and $BaCl_2$ is decreased. This argues against the rate concept as a declaration of this desensitisation (Huidobro & Valetta, 1961; Paton, 1961). With lower doses of acetylcholinomimetics, but doses high enough to produce the maximal isotonic response, no desensitisation is observed after washing till the basal tone returns.

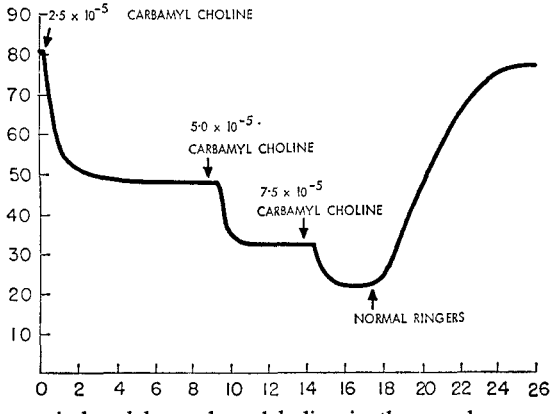


FIG. 10. Changes induced by carbamylcholine in the membrane potential of the isolated electroplax. Note after application of the drug there is a steady increase of the depolarization approaching some equilibrium state. There is no fade phenomenon in the curves (Higman, Pollewski & Bartels, 1963).

The phenomenon of the fade in the response and the specific desensitisation or tachyphylaxis has been considered earlier. The interpretation of this was the aim of the "potential" theory on drug action, a model introduced by Straub (1907), and discussed by Clark (1937a). In this model the drug is supposed to act only as long as there is a concentration gradient from the extracellular to the intracellular phase, or in other words as long as there is a net flux of drug into the cell or to the receptors. This model, too, allows for an initial maximum in the time-effect curve and for the fade in the effect with the decrease of the gradient or flux. The effect may totally fade away although the drug is still present. The model also predicts a specific desensitisation of the biological object after removal of the drug from the extracellular phase as long as there is an intracellular

residue of the drug. Mackay (1963) recently enlarged the scope of the "potential" theory by introducing specific carriers for the transportation or flux of the drug, which implies a combination of "potential" theory and receptor theory.

Structure and action

If the induction of a biological effect is the resultant of the interaction between the drug molecules and specific receptors, a relation between the chemical structure, or better the physical and chemical properties of the drug, and the activity must exist. Nevertheless, often, such a relation is not observed. There are two main reasons for this failure.

LIMITATIONS OF KNOWLEDGE ABOUT STRUCTURE

Structural formulae are a very poor means for expressing the physico-chemical properties of molecules. Drugs apparently similar on the basis of their structural formulae may differ essentially in their properties. The reverse is also true. Drugs apparently different in structure may be identical in their essential physico-chemical properties.

LIMITATIONS OF KNOWLEDGE ABOUT ACTION

The biological activities of the compounds compared may look identical from a crude phenomenological point of view; nevertheless, they may differ essentially in their mechanism of action. Consider for instance, the group of muscle relaxants, the group of convulsants or the group of diuretics. In principle, only drugs which induce their biological actions on identical receptors may be compared. Moreover, differences in transport and drug metabolism, for instance, have to be taken into account.

AFFINITY AND INTRINSIC ACTIVITY

As a consequence of the differentiation between affinity and intrinsic activity one may try to differentiate within the structure of the molecule between those moieties mainly concerned with the intrinsic activity and those concerned with the affinity. The experimental results represented in the Figs 2–5 suggest that, for the parasympathomimetic action of acetylcholine and its derivatives, the properties of the onium group are essential for the intrinsic activity, while the chain is mainly of importance for the affinity to the receptors. As a matter of fact the onium group also contributes to the affinity.

What is the significance of the ester group in acetylcholine and its equivalents in its various analogs for the muscarinic action? As a rule an equivalent is found in these analogs for the ether-oxygen but not for the carbonyl group of the ester moiety. This indicates that for muscarinic activity, the ether-oxygen is more concerned with the drug-receptor interaction than the carbonyl group.

The study of the influence of gradual ethylation of the onium group in acetylcholine and its various analogs tested on the isolated gut of the rat

as is shown in Table 1 (Simonis, Ariëns & Rodrigues de Miranda, 1964) reveals an interesting aspect. The change from agonist to competitive antagonist in the course of the ethylation is retarded by the presence of the ester moiety or its substitutes in the parasympathomimetics. In acetylcholine itself the triethyl derivative still acts as a full agonist, although the affinity is decreased. The conclusion may be that the ester moiety and its substitutes are not essential for the intrinsic activity, but nevertheless influence this parameter. It may be that a mutual neutralisation or ion-pair formation between the onium group and a complementary anionic site on the receptors is essential for the induction of the stimulus and therefore for the production of the effect. The interaction between the ester moiety and its complement on the receptor then appears to have a facilitating action as far as this neutralisation or ion-pair formation is concerned. In the absence of the facilitating ester group, the ethylation of the onium group, causing steric hindrance or changes in charge distribution, more easily interferes with a true neutralisation or ion-pair formation. The cationic head after ethylation may still be attracted by the anionic site, thus shielding this part of the receptor; the drug then acts as a blocking compound, a competitive antagonist. The ethylated molecule then takes over the place but not the function of acetylcholine.

"ATTACHING" AND "ACTING" MOIETIES

One must be aware of the fact that, as mentioned, drug-receptor interaction implies a mutual moulding of drug and receptor. The usual model of key and lock is much too static. The interaction of the ester moiety in acetylcholine and its complement with the receptor may well change the conformation or charge distribution of the receptor in such a way that the approach between the cationic head of the drug and the anionic site on the receptor is facilitated. The consequence is that, although in a study on the relation between structure and action certain moieties of the drug molecule are found to be of special importance for the intrinsic activity and other moieties for the affinity, as a rule this differentiation will not be sharp, since the various moieties are parts of the molecule as a whole and therefore interdependent.

Cavallini, Massarani, Nardi & Mauri (1961) differentiated a supporting moiety, mainly conferring to the drug affinity for the receptors, and a radical moiety determining the type of action. An analogous differentiation is made in the field of cancer chemotherapy. In the various nitrogen-mustard derivatives there can be differentiated the mustard moiety, the cytoxic group of the "war-head" and the carrier (Ross, 1962). Groups such as amino-acids, sugars and steroids (Rao & Price, 1962) may act as carrier moieties.

Especially in larger drug molecules, like the biologically active polypeptides, a distinction between moieties essential for the intrinsic activity and moieties mainly contributing to drug binding, to the affinity, seems feasible. Take for instance the differentiation in an "attachment site" and "active site" or a differentiation in "functional amino- acid residues"

and "filler sequences" in the various polypeptide hormones as postulated by Hofmann (1960) and Schwyzer (1962, 1963). The differentiation of a "Haft Gruppe" and a "Wirk Gruppe" in vitamins as postulated by Martius (1955, 1958) is also reminiscent of affinity and intrinsic activity. The various examples given have much in common with the differentiation between a haptophoric group and a toxophoric group in drugs already postulated by Ehrlich, which also implies a differentiation between affinity and intrinsic activity.

Receptors

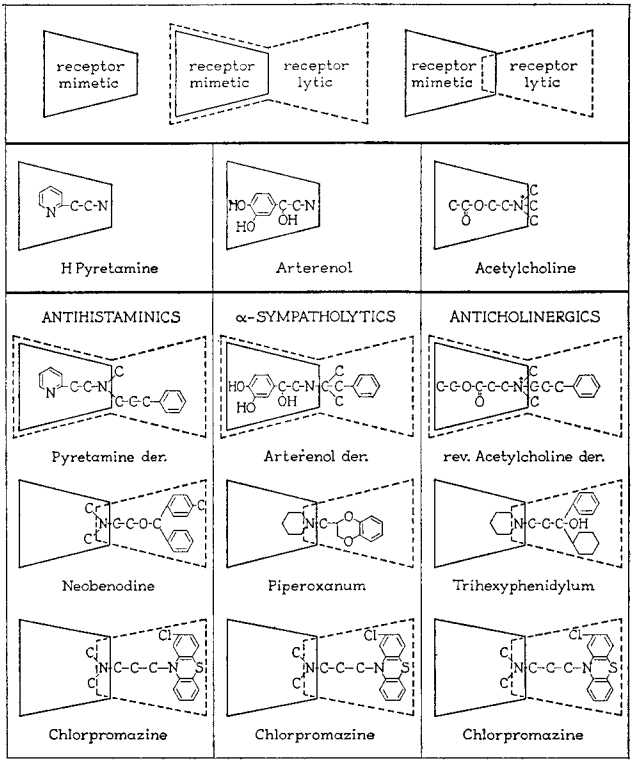
The main if not the only source of information on the properties of the receptors is the study of structure-activity relations of drugs. This study allows us, although on a basis of indirect evidence, to develop certain views on the still purely hypothetical but, for the molecular approach to drug action, indispensable receptors.

AFFINITY TO "COMMON" RECEPTORS

As mentioned before, agonist and competitive antagonist act on a common receptor. As will be shown this does not imply an action on strictly identical receptors (Ariëns & Simonis, 1960; Ariëns, 1962a, 1964). Introduction of substituents gradually increasing in length on the amino- or onium group of compounds such as histamine, arterenol (noradrenaline) and acetylcholine, or on the acetic acid group in acetylcholine results in a gradual change from agonists to competitive antagonistic compounds. The intrinsic activity, but also the affinity strongly decreases. The lytics obtained have a low affinity to the receptors. With the introduction of larger groups, especially groups with planar rings such as aralkyl groups, the affinity strongly increases again and highly active lytics are obtained. Tables 2 and 3 give examples of such series of compounds. The conclusion may be that the lytic compounds thus obtained are dependent for much of their affinity not on the original receptor for the mimetic but on additional receptor parts.

Histamine, acetylcholine and noradrenaline are stimulants bearing strong polar groups. Their receptors, being complementary to these stimulant drugs, will also present concentrations of strong polar groups. In the vicinity more indifferent, less-polar surface areas may be expected. These areas may serve for the interaction of the ring-bearing substituents mentioned above. Many of the lytic drugs probably interact slightly with the receptor area of the agonist, the mimetic, but mainly their action will be on the adjacent, additional, more indifferent receptor area. Probably histaminomimetics, acetylcholinomimetics and sympathomimetics have in common with many of their respective competitive antagonists only the anionic site in their receptors. This makes it understandable that most mimetics show little or no chemical relation to the corresponding lytics, and that certain lytics such as for example chlorpromazine, have the ability "to block the receptors" for different types of mimetics. These relations are represented in a schematic way in Table 4 (Ariëns & Simonis, 1960; Ariëns,

TABLE 4. MIMETICS (AGONISTS) AND DIFFERENT TYPES OF LYTICS (COMPETITIVE ANTAGONISTS) IN RELATION TO THEIR HYPOTHETICAL RECEPTOR SURFACES



Note: chlorpromazine has an antihistaminic, an anti-adrenergic and an anticholinergic action.

1962a, 1964). Introduction of certain, especially spatial, properties in such multipotent lytics may result in an increase in specificity again (Harms, 1956; Harms & Nauta, 1960), which is feasible, since the indifferent surface areas adjacent to the receptors for histamine, acetylcholine and noradrenaline will not be identical and may differ especially in spatial relations.

The loss of binding capacity to the original receptor areas and the acquisition of new binding capacities on additional receptor parts, as demonstrated in the series of acetylcholine derivatives (Table 2), has interesting consequences if the esters of β-methylcholine are studied. As is well-known, both stereoisomers of acetyl-β-methylcholine differ strongly in their biological activity. For the highly active competitive antagonist obtained by esterification of β-methylcholine with for instance 2,2′-diphenyl-2″-hydroxyacetic acid (benzilic acid) there is hardly any difference in potency between the two stereoisomers. If a centre of asymmetry is introduced in the acidic moiety, for instance by esterification of β-methylcholine with 2-phenyl-2′-cyclohexyl-2″-hydroxyacetic acid highly active lytics are obtained again. Now a large difference in potency

is found between the stereoisomers which differ in the steric configuration of the acidic moiety of the molecule, while only small differences in potency are observed for those stereoisomers that differ in the configuration of the β-methylcholine moiety (Ellenbroek, 1964).

Such relations are expected on a basis of the shift of the binding capacity from the cholinic to the acidic moiety of the molecule. On the other hand these results demonstrate that the significance of a centre of asymmetry for the activity of a drug molecule is strongly dependent on the degree to which the moiety of the molecule in which the centre of asymmetry is located, contributes to the binding of the drug to the receptor or to the activity of the drug. This puts restrictions on the rule that the differences in activity of stereoisomers is large for highly active and small for less active compounds, known as Pfeiffer's rule (1956) (Beckett, 1963).

ACTION ON DIFFERENT RECEPTORS

The above reasoning dealt with the interaction of drug and receptor as far as the affinity is concerned. The drug-receptor interaction is also concerned with the intrinsic activity. The catecholamines are interesting in this respect. Drugs like adrenaline induce effects on two types of receptors called α- and β-receptors (Ahlquist, 1959). Substitution of large alkyl or aralkyl groups on the amino-group of noradrenaline results in a loss of the intrinsic activity on the α-receptors. The compounds obtained act as α-sympatholytics and β-sympathomimetics simultaneously. If on the other hand the catechol configuration is eliminated such as is the case in dichloroisoprenaline and in pronethalol, the intrinsic activity on the β-receptors is lost and β-sympatholytics are obtained (Ariëns & Simonis, 1960; Howe, 1963). A close correlation of structure and action is found in the series of *N*-alkyl or -aralkyl substituted noradrenalines (β-sympathomimetics) and *N*-alkyl or -aralkyl substituted pronethalols (β-sympatholytics). In both series, branched alkyl groups such as isopropyl and t-butyl result in highly active compounds. The same obtains for the introduction of a phenyl-isopropyl group. The absolute steric configuration of the most active isomers of isoprenaline (mimetics) and dichloroisoprenaline and the isopropyl derivative pronethalol (lytics) are identical (Howe, 1963). These relations make it highly probable that these β-sympatholytics really block in a more strict sense the surface of the β-receptors. The fact that blockade of the β-receptors by β-sympatholytics such as pronethalol does not interfere with the induction of effects by α-sympathomimetics on the α-receptors strongly argues for two really different entities as far as α- and β-receptors are concerned.

Further, the relationships described indicate that the interaction between the cationic amino-group in the catecholamines and some complementary anionic site on the α-receptor is essential for the induction of the effect, or for the intrinsic activity there. On the β-receptors, for the induction of the effect, or the intrinsic activity, the interaction between the catechol group and the receptor appears to be essential. Analogous

reasoning can be given for the relations between muscarinic and nicotinic drugs and their receptors, and relations between the polypeptide hormones related to oxytocin and ADH and their receptors (Schwyzer, 1963).

ISOLATED RECEPTORS

Various investigators (Chagas, Penna-Franca, Hassón, Crocker, Nishie & Garcia, 1958; Hassón & Chagas, 1959; Ehrenpreis, 1962a, 1963b) tried to isolate receptors and studied the interaction of drugs with such isolated "receptor proteins" or receptor substances.

A characteristic of the receptors on which effects are induced by stimulant drugs is that the changes induced by the drug in charge distribution and shape of the receptor, are propagated to surrounding molecules. This then leads to a stimulus and consequently to the effects. This implies an intricate relationship between the receptor molecule and the adjoining molecules which is essential for the properties of the receptor *in situ*. The isolated receptor will, as a rule, be essentially changed in its charge distribution and conformation as compared to the receptor *in situ*. Although it may still bind drugs, the isolated receptor will differ in essential aspects from the receptor *in situ*. Certainly no stimulus can be induced on the "isolated receptor". Drug binding to macromolecules obtained from various tissues can serve as a model for the study of the interaction between drugs and receptors in general (Hassón & Chagas, 1959; Ehrenpreis, 1962b, 1963a).

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