J. Pharm. Pharmacol., 1964, **16**, 289–312

Review Article

A molecular basis for drug action. The interaction of one or more drugs with different receptors*

E. J. ARIËNS AND A. M. SIMONIS

ARIENS & Simonis (1964) in the preceding review paid special attention to the action of combinations of drugs which interact with common receptors, the competitive interaction. In the following sections other kinds of drug-receptor interaction will be discussed.

NON-COMPETITIVE INTERACTION

Non-competitive interaction is the interference by a drug B with the action of a drug A, both drugs acting on different, but interrelated receptors, while drug B is inactive if applied alone and only modifies the action of a second drug, A. This type of interaction can be put into a formula based on the mass-action law (eqn 1 is an example). The second term in this equation determines the change in the effect of drug A brought about by drug B.

$$\begin{array}{c} E \\ | \\ R\ R' \\ \uparrow\downarrow\ \ \uparrow\downarrow \\ A\ \ B \end{array} \qquad E_{AB'}/E_m = E_A/E_m\left(1+\frac{\beta'}{1+\frac{K'_B}{[B]}}\right)=\frac{\alpha}{1+\frac{K_A}{[A]}}\left(1+\frac{\beta'}{1+\frac{K'_B}{[B]}}\right) \qquad (1)$$

(compare with eqn 2, Ariëns & Simonis, 1964).

The result of the interaction of both drugs here also depends on the intrinsic activities of A and B, α and β respectively. A well-known example of the non-competitive interaction is the non-competitive antagonism (Ariëns, Simonis & de Groot, 1955; Ariëns, Van Rossum & Simonis, 1956; Ariëns & Simonis, 1961; Ariëns, 1964). Then the intrinsic activity β' of B is less than zero, e.g., -1. The effect of A ($\alpha > 0$) is reduced by the presence of B. If β' is greater than zero, B acts as a sensitiser for A. Then the effect of A is increased by the presence of B.

With a non-competitive antagonism the presence of the antagonist B results in a decline in the curves for the agonist A. An increase in the dose of A cannot overcome the influence of B. The antagonism is insurmountable. This is contrary to the case of the competitive inhibition

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* The second of two reviews on 'A molecular basis for drug action'. The first review appeared in the March, 1964 issue of the Journal, pp. 137–157.

described earlier (Ariëns & Simonis, 1964). The musculotropic spasmolytics of the papaverine-type behave as non-competitive antagonists for many spasmogens (Fig. 1).

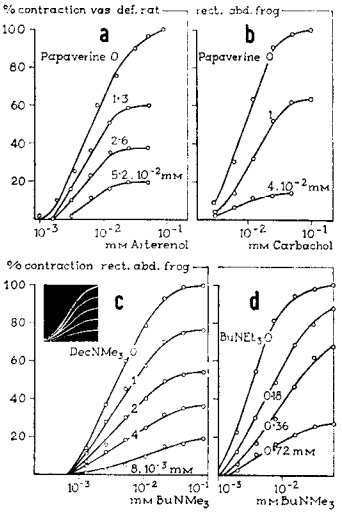


FIG. 1a–d. Cumulative log concentration-response curves for various agonists and the influence thereon of different antagonists: (a) arterenol (noradrenaline) with papaverine, (b) carbachol with papaverine, (c) $BuNMe_3$ with $DecNMe_3$ and (d) $BuNMe_3$ with $BuNEt_3$. Note the decline in the curves which indicates a non-competitive antagonistic action. Compare the experimental curves with the set of theoretical curves (calculated from eqn 1) inset.

In the earlier review (Ariëns & Simonis, 1964) the gradual change from agonist to competitive antagonist in an homologous series of compounds was described. Examples of homologous series of compounds are known in which a gradual change takes place from active compounds to "inactive" compounds which turn out behave as non-competitive antagonists of the active ones. In a series of alkyltrimethyl-ammonium derivatives ($AlkNMe_3$) tested on the isolated rectus abdominis muscle of the frog, the compounds $BuNMe_3$ and $PentNMe_3$ act as agonists while $DecNMe_3$ behaves as their non-competitive antagonist (see Fig. 1c). In the series of $BuNR_3$ derivatives $BuNMe_3$ acts as an agonist while $BuNEt_3$ acts as its non-competitive antagonist (see Fig. 1d) (Ariëns & others, 1955, 1956a; Ariëns & van Rossum, 1957; Ariëns, 1964).

The activity of the non-competitive antagonist is expressed as the pD'_2 value; this is the negative logarithm of that molar concentration of the antagonist that brings about a reduction in the effect of the agonist equal to 50% of the maximal reduction that can be brought about by high doses of the non-competitive antagonist (Ariëns & van Rossum, 1957; Ariëns, 1964).

The various non-competitive antagonists of a certain agonist do not necessarily act on the same receptors. They may interfere with the induction of the stimulus or the evocation of the effect at various points in

the sequence of events initiated by the interaction of the stimulant drug, the agonist, and its specific receptors. The changes brought about by the non-competitive antagonists in the response of the agonist simulate a change in the intrinsic activity.

Another interesting type of non-competitive interaction is the change in the affinity between the agonist and its receptors as a result of the action of a second drug on receptors different from those for the agonist. This type of non-competitive interaction, which in some ways is reminiscent of the competitive interaction, is discussed further by Ariëns & others (1956a) and Ariëns (1964).

The displacement of oxygen by carbon monoxide from the haem moiety of haemoglobin provides a model for the concept of competition for common receptors. There is another aspect: after binding of oxygen to two of the four haem groups the binding of carbon monoxide to the other haem groups is facilitated. In the same way carbon monoxide facilitates the binding of oxygen. This is an example of a change in the affinity of a compound to the receptors induced by another compound on other receptors (Pauling, 1935; Roughton & Darling, 1944; Roughton, Legge & Nicholson, 1949; Roughton, 1949). For further information the reader is referred to the literature (Ariëns, 1964).

DRUGS WITH A MULTIPLE ACTION

In the series of $AlkNMe_3$ derivatives and $BuNR_3$ derivatives tested on the rectus abdominis muscle of the frog mentioned above, the loss of the activity is not caused by a decrease in the intrinsic activity but by the introduction of non-competitive inhibitory properties as a result of the change in the chemical structure of the compounds.

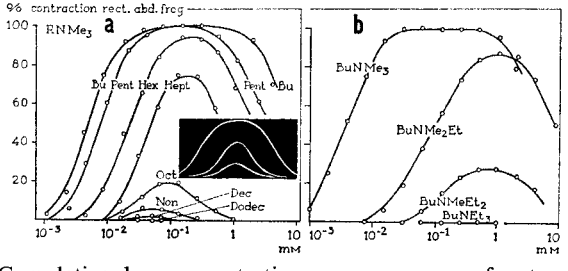
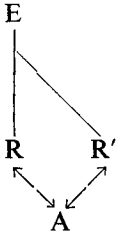


FIG. 2a, b. Cumulative log concentration-response curves for two homologous series of compounds, namely $RNMe_3$ derivatives and $BuNR_3$ derivatives. Note the gradual change from active to "inactive" compounds, the latter being non-competitive antagonists of the active compounds (see Fig. 1c and d respectively). Compare the experimental curves with the set of theoretical curves (calculated from eqn 2) inset.

In the transition from agonist to non-competitive antagonist, compounds with a multiple action are found: compounds which at lower doses behave as agonists while at higher doses, by interference of the non-competitive component in the action, the effect originally induced is abolished (see Fig. 2).

An equation for this type of non-competitive auto-inhibition (eqn 2) is obtained by substituting in eqn 1 α', K'_A and [A] for β', K'_B and [B]

respectively, while the intrinsic activities are given values of $\alpha = 1$ and $\alpha' = -1$. If in eqn 2 $\alpha = 1$ and $\alpha' > 0$, the compound A exhibits an auto-sensitisation (Ariëns & others, 1956a; Ariëns, 1964).



$$E_{AA'}/E_m = \frac{\alpha}{1 + \frac{K_A}{[A]}}\left(1 + \frac{\alpha'}{1 + \frac{K'_A}{[A]}}\right) \quad .. \quad (2)$$

In the series of $AlkNMe_3$ derivatives, the gradual elongation of the alkyl chain results in a gradual decrease of K'_A/K_A and an enhancement of the auto-inhibition. With the decrease in K'_A/K_A the auto-inhibitive properties of the drug will manifest themselves before the maximum effect of A possible with the object concerned, E_{Am}, is reached. The intermediate compounds for which this occurs may also be called partial agonists. Examples are the compounds $HexNMe_3$ and $HeptNMe_3$ in Fig. 2a and $BuNMe_2Et$ and $BuNMeEt_2$ in Fig. 2b. They differ from the agonistic compounds such as $BuNMe_3$ and $PentNMe_3$ in the slope of their curves and in the maximal effect obtained with them. These intermediate compounds interact with two different types of receptors simultaneously; they have a multiple action. They differ essentially from the type of partial agonists found on the transition from agonists to competitive antagonists (see Ariëns & Simonis, 1964). For very low values of K'_A/K_A the compounds will behave as pure non-competitive antagonists. Because of the relatively high affinity, $1/K'_A$, to the receptors, R', these compounds will saturate the receptors R' in doses which induce no effect on the receptors R. The compounds $DecNMe_3$ and $BuNEt_3$ are examples (see Fig. 1c and 1d).

If a partial agonist with a multiple action of the type just described such as $HeptNMe_3$ and $BuNMe_2Et$, is combined with a competitive

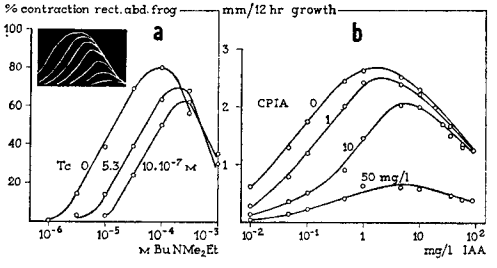
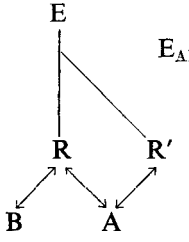


FIG. 3a, b. Cumulative log concentration-response curves for two compounds exhibiting an auto-inhibition and the influence thereon of various concentrations of a competitive antagonist: (a) $BuNMe_2Et$ and the influence of tubocurarine, and (b) 3-indolyl acetic acid (IAA) tested on the growth of Avena coleoptile sections and the influence on it of 4-chlorophenoxy-isobutyric acid (CPIA) (after Foster & others, 1955). Note the parallel shift in the ascending part of the curves. Compare the experimental curves with the set of theoretical curves (calculated from eqn 3) inset.

antagonist acting on the receptors on which the agonistic effect is induced, only the ascending part of the bell-shaped curve is expected to shift. The drug-receptor interaction responsible for the descending part is not interfered with by such a competitive antagonist. The equation for this type of interaction, eqn 3, is obtained from a combination of eqn 3, in Ariëns & Simonis (1964) and eqn 2 above. The intrinsic activities have to be substituted then as $\alpha = 1$, $\beta = 0$ and $\alpha' = -1$. Fig. 3 gives experimental curves for this type of interaction (Ariëns & others, 1955, 1956a; Foster, McRae & Bonner, 1955; Ariëns, 1964).



$$E_{ABA'}/E_m = \left(\frac{\alpha}{1+\left(1+\frac{[B]}{K_B}\right)\frac{K_A}{[A]}} + \frac{\beta}{1+\left(1+\frac{[A]}{K_A}\right)\frac{K_B}{B}}\right)\left(1+\frac{\alpha'}{1+\frac{K'_A}{[A]}}\right) \quad .. \quad .. \quad (3)$$

Another interesting combination of drugs is that of a partial agonist such as $HexNMe_3$ and $HeptNMe_3$ (Fig. 4a) or $BuNMe_2Et$ and $BuNMeEt_2$ (Fig. 4b) with a non-competitive antagonist, e.g., $DecNMe_3$ or papaverine.

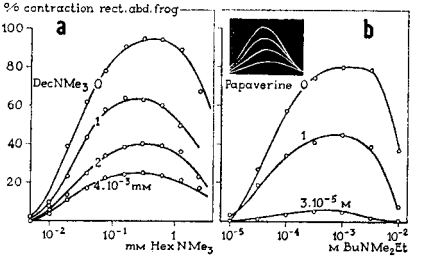
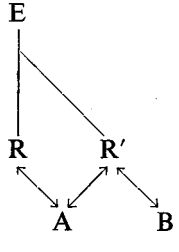


FIG. 4a, b. Cumulative log concentration-response curves for two compounds exhibiting an auto-inhibition and the influence thereon of various concentrations of a non-competitive antagonist: (a) $HexNM_3$ and (b) $BuNMe_2Et$, in the presence of $DecNM_3$ and papaverine respectively. Note the decline in the curves. Compare the experimental curves with the set of theoretical curves (calculated from eqn 4) inset.

Eqn 4, which represents this type of interaction, results from a combination of eqn 3 (Ariëns & Simonis, 1964) and eqn 2 above. In eqn 4 $\alpha = 1$, $\alpha' = -1$ and $\beta' = -1$ are substituted for the intrinsic activities. Fig. 4 gives experimental curves for this type of interaction.



$$\frac{E_{AA'B'}}{E_m} = \frac{\alpha}{1+(K_A/[A])}\left[1+\left(\frac{\alpha'}{1+(1+[B]/K'_B)K'_A/[A]} + \frac{\beta'}{1+(1+[A]/K'_A)K'_B/[B]}\right)\right] \quad .. \quad (4)$$

Attention is drawn to the Figs. 6 and 7 in the preceding review (Ariëns & Simonis, 1964) which represent the hydrolysis of various esters of choline and of combinations of such esters by acetylcholinesterase. The substrate inhibition manifested by the dose-effect curves in those figures, which was not considered then, now requires special attention. The curves in both figures represent enzymological examples of dose-effect curves which are clear-cut analogues for the curves represented in Figs 2a, 2b, and 3a and 3b of this text respectively. For most of the types of dose-response curves described in this paper analogous examples are known in enzymology (van Rossum & Hurkmans, 1962; Webb, 1963).

The experimental results presented agree well with the theory as can be seen from the equations and the theoretical curves calculated on basis of these equations.

If a compound is qualified as "inactive" it is necessary to specify the type of inactivity. The "inactive" compounds may have interesting properties such as competitive or non-competitive antagonistic actions towards related agonists. For the chemist designing new compounds it may be especially useful to know whether the loss in activity as a result of certain chemical modifications is based on a loss in affinity, a loss in intrinsic activity or the introduction of non-competitive antagonistic properties. The analysis of dose-response curves for combinations of drugs is required for this purpose.

The equations given in the various sections are presented in such a way that the application of the mass-action law to the various drug-receptor interactions involved in the actions of the drugs and drug

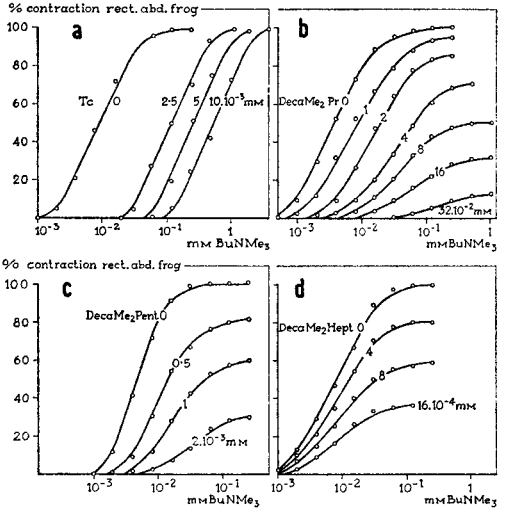
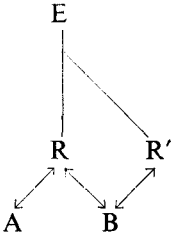


FIG. 5a–d. Cumulative log concentration-response curves for $BuNMe_3$ and the influence thereon of various concentrations of antagonistic compounds: (a) tubocurarine, (b) $DecaMe_2Pr$, (c) $DecaMe_2Pent$ and (d) $DecaMe_2Hept$. Note the purely competitive antagonistic action of tubocurarine and the gradual change from the dualism in antagonism of $DecaMe_2Pr$ to the purely non-competitive antagonistic action of $DecaMe_2Hept$.

combinations is clearly expressed. As well as the type of interaction discussed in detail here, these equations cover many other types of action of drugs and drug combinations if suitable values for the various constants, the affinities and the intrinsic activities, are introduced.

DRUGS WITH A MULTIPLE ANTAGONISTIC ACTION

In the foregoing sections, drugs which are intermediates between agonists and competitive antagonists such as pentylNMe_2Et (see Fig. 5, Ariëns & Simonis, 1964) and drugs which are intermediates between agonists and non-competitive antagonists such as heptylNMe_3 and butylNMe_2Et (see Fig. 2) , have been discussed. But there also exist drugs which are intermediates between competitive and non-competitive antagonists. These are compounds with a competitive antagonistic action on which a non-competitive antagonistic action is superimposed. If in eqn 5, which is closely related to eqn 3, $\alpha = 1$, $\beta = 0$ and $\beta' = -1$ is substituted for the intrinsic activities, the equation represents the interaction of an agonistic compound A with a compound B having a multiple antagonistic action such as that just described (Ariëns & others, 1956a, 1957; Ariëns & Simonis, 1961; Ariëns, 1964).



$$\frac{E_{ABB'}}{E_m} = \left(\frac{\alpha}{1+\left(1+\frac{[B]}{K_B}\right)\frac{K_A}{[A]}} + \frac{\beta}{1+\left(1+\frac{[A]}{K_A}\right)\frac{K_B}{[B]}}\right)\left(1+\frac{\beta'}{1+\frac{K'_B}{[B]}}\right) \quad .. \quad .. \quad (5)$$

Many examples of compounds with a multiple antagonistic action are known. This is so, for instance, in the series of the bis-*N*-alkyl-substituted decamethonium derivatives, where the propyl and the pentyl compounds (Fig. 5b and c) are intermediates between curariform drugs of the curarimimetic type, such as tubocurarine (Fig. 5a) and triethylsuccinylcholine, and curariform drugs of a practically pure non-competitive type

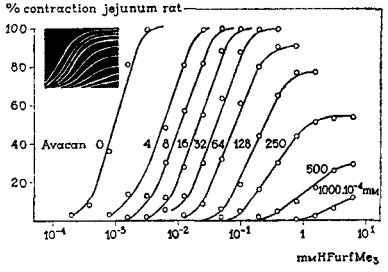


FIG. 6a. Cumulative log concentration-response curves for the agonist furtrethonium (HFurfMe_3) and the influence thereon of various concentrations of Avacan. Note the competitive component in the action of Avacan (the parallel shift) on which a non-competitive component (the decline in the maximal response and the slope of the curves) is superimposed. Compare the experimental curves with the set of theoretical curves (calculated from eqn 5) inset.

such as the bis-*N*-heptyl derivative of decamethonium (see Fig. 5d) (van Rossum & Ariëns, 1959a; Ariëns, 1964). Other examples are the compounds in which a parasympatholytic and a papaverine-like spasmolytic action are combined, for instance, Avacan (isopentyl α-(2-diethylamionethylamino)phenylacetate hydrochloride), and adiphenine (Goodman & Gilman, 1955; Formanek & Weis, 1963). Figs. 6a and 6b represent experimental dose-response curves obtained with Avacan.

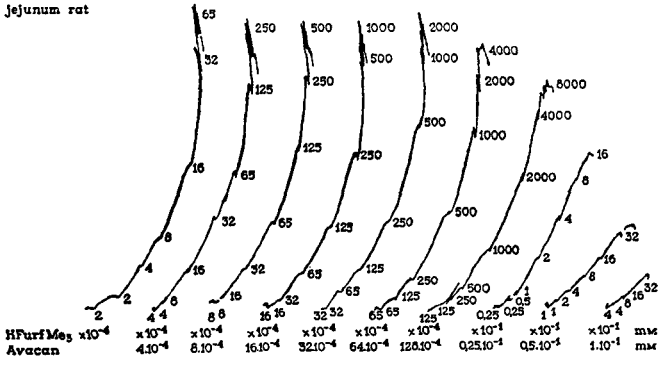


FIG. 6b. A registerogram of cumulative dose-response curves for a combination of drugs as represented in Fig. 6a.

This and the foregoing sections on non-competitive interaction dealt with the interactions of drugs with different but interdependent receptors R and R′, such that the effect induced on one type of receptor, R, is changed by the action of drugs on the second type, R′, while on the receptor R′ no primary effect can be induced. The following section will deal with the action of drugs on different but interdependent receptors R_I and R_{II} such that an effect can be induced on each of the receptors independently. This type of drug-receptor interaction can be considered as a functional interaction to distinguish it from the non-competitive interaction.

COMBINATIONS OF DRUGS WHICH INTERACT WITH DIFFERENT RECEPTORS, BUT PRODUCE THEIR EFFECT BY MEANS OF A COMMON EFFECTOR SYSTEM

In the most simple case the effect of the combination of two agonists A and B will be equal to:

$$\begin{array}{c} E \\ | \\ R_I \quad R_{II} \\ \updownarrow \quad \updownarrow \\ A \quad B \end{array} \quad \frac{E_A}{E_m} + \frac{E_B}{E_m}\left(1 - \frac{E_A}{E_m}\right) = \frac{E_B}{E_m} + \frac{E_A}{E_m}\left(1 - \frac{E_B}{E_m}\right) = \frac{E_A}{E_m} + \frac{E_B}{E_m} - \frac{E_A E_B}{E_m^2} \qquad (6)$$

where E_A/E_m and E_B/E_m are the effects of [A] and [B] if applied singly. The term $E_A E_B/E_m^2$ represents the mutual hindrance of the drugs as far as the use of the common effector-system is concerned. If $E_m^2 \gg E_A E_B$

the effect of the combined drugs will be close to the sum of the effects of the single drugs (Ariëns & others, 1956b, 1957; Ariëns, 1964).

Fig. 5 in the preceding review (Ariëns & Simonis, 1964) represents the combination of a partial agonist and another agonist both inducing their effects on the same receptors. The displacement of the agonist by the partial agonist results in an antagonistic effect. What then will be the consequence of combining such a partial agonist with an agonist which induces its effect on different receptors but by means of the same effector system? There is no mutual displacement of the drugs now. The partial agonists will always contribute to the effect independent of the dose of agonist used. Fig. 7 gives examples of such a combination.

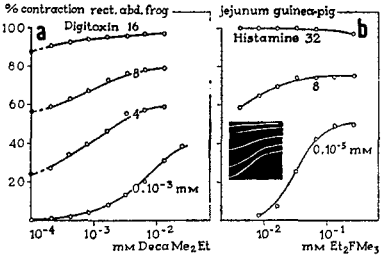


FIG. 7a, b. Cumulative log concentration-response curves for two partial agonists and the influence thereon of various concentrations of other agonists: (a) $DecaMe_2Et$ in combination with digitoxin and (b) Et_2FMe_3 in combination with histamine. Note the absence of a dualism in effect for the partial agonist which indicates a functional interaction between the compounds combined. (Compare with Fig. 5, in preceding review, p. 142). Compare the experimental curves with the set of theoretical curves (calculated from eqn 6) inset.

The spasmogen, bis-mono-ethyl-substituted decamethonium, a partial agonist, is combined with another spasmogen, digitoxin. These compounds interact with different receptors. The decamethonium derivative is antagonised in a competitive way by tubocurarine for example, while digitoxin is not antagonised by this drug. As expected, the partial agonist always acts synergistically in this combination (Fig. 7a) (Ariëns & others, 1956a). If the decamethonium derivative is combined with succinylcholine, a spasmogen which is also inhibited competitively by tubocurarine, a set of curves of the type represented in Fig. 5 of the preceding review is obtained (Ariëns & Simonis, 1964). Another example is given in Fig. 7b. Histamine acts on receptors different from those for the parasympathomimetic compound 2,2-diethyl-4-(trimethylammonium)methyl-1,3-dioxolane (Et_2FMe_3), a partial agonist. If combined with histamine, Et_2FMe_3 only acts as a synergist (van Rossum & Ariëns, 1959b).

Another example of the combination of drugs acting on different receptors but via a common effector system is the combination of an agonist with two antagonistic drugs, one of which is a competitive antagonist of the agonist while the other one is not.

As a basis for a comparison, the combination of an agonist with two competitive antagonists will be discussed first. Equation 7 represents

the competitive interaction of three compounds, A, B and C, with common receptors. If in this equation $\alpha = 1$, $\beta = 0$ and $\gamma = 0$ it represents the combination of an agonist and two competitive antagonists.

$$E_{ABC}/E_m = \frac{\alpha}{1 + \left(1 + \frac{[B]}{K_B} + \frac{[C]}{K_C}\right)\frac{K_A}{[A]}} + \frac{\beta}{1 + \left(1 + \frac{[A]}{K_A} + \frac{[C]}{K_C}\right)\frac{K_B}{[B]}} + \frac{\gamma}{1 + \left(1 + \frac{[A]}{K_A} + \frac{[B]}{K_B}\right)\frac{K_C}{[C]}} \quad \ldots \quad \ldots \quad (7)$$

If equipotent doses of the parasympatholytics lachesine and atropine are combined and tested against the parasympathomimetic furtrethonium, the effect of this combination is equal to the effect of doubling the separate doses (Fig. 8a). This is expected on basis of the theory represented by eqn 7. For the combination of a potent dose of e.g. lachesine with a less potent dose of atropine, the experimental results also agree well with the theory (Fig. 8c).

This type of co-operation between two drugs acting on common receptors, here the competitive antagonists B and C, is known as an additive action. An additive action also occurs if the drugs A and B, in eqn 3 of the preceding review (see page 141), have equal intrinsic activities, e.g. such that $\alpha = \beta = 1$; this implies that both drugs are agonists. This means that on basis of equipotencies, where $K_A/[A] = K_B/[B]$, or the effect of the dose of drug A is equal to the effect of the dose of drug B, either drug can be substituted by the other without a change in the effect. This relationship is represented in eqn 8. In case of an additive action, $q = 1$, independently of the value of n, which may vary from 1 to ∞.

$$q = \frac{\text{effect of } \left[\frac{1}{n}[A] + \left(1 - \frac{1}{n}\right)[B]\right]}{\text{effect of } [A]} \quad \ldots \quad \ldots \quad (8)$$

This can easily be seen by combining eqns 2 and 3 in the preceding review (pages 139 and 141), which gives eqn 9.

$$q = E_{AB}/E_A = \frac{(1 + K_A/[A])\,(\alpha[A]K_An + \beta[B]/K_B - \beta[B]/K_Bn)}{\alpha(1 + [A]/K_An + [B]/K_B - [B]/K_Bn)} \quad (9)$$

For the case where $[A]/K_A = [B]/K_B$ and $\alpha = \beta$, $E_{AB}/E_A = q$ becomes 1.

If $q > 1$, the effect obtained with the combination of A and B is larger than that expected in the case of an additive action, then there is potentiation. If $q < 1$ the effect obtained with the combination of A and B is smaller than in the case of an additive action. These relations hold true also for combinations of an agonist and a partial agonist acting on common receptors (Ariëns & others, 1956b; Ariëns & van Rossum, 1957a; Ariëns, 1964).

A comparison of the effect of a combination of two competitive antagonists such as atropine and lachesine, with the effect of a combination of a competitive antagonist, for instance, lachesine and an antagonist

such as isoprenaline (isopropylarterenol), often called a functional antagonist, clearly demonstrates the difference between the additive action for the first type of combination (Fig. 8a, c) and the potentiation arising from the second type (Fig. 8b, d). In the case of a combination of lachesine with isoprenaline the effect comes close to the sum of the effects of the single doses.

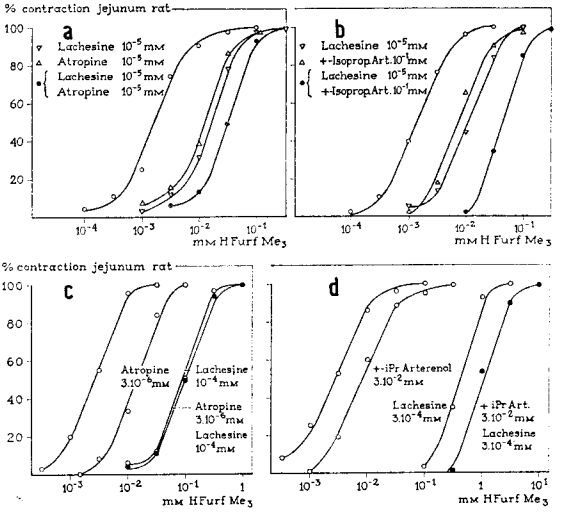


FIG. 8a–d. Cumulative log concentration-response curves for the parasympathomimetic furtrethonium ($HFurfMe_3$) and the influence thereon of the competitive antagonists lachesine and atropine and combinations of these two drugs (a and c), and the influence of the competitive antagonist lachesine and the functional antagonist +-isopropylarterenol (isoprenaline) and the combination of these two drugs (b and d). (a,b) Note, with the combination of lachesine and atropine, there is much less than a summation of the antagonistic actions of the single compounds. With the combinations of lachesine and isoprenaline there is nearly a summation of the antagonistic actions of the single compounds. (c,d) Note the doses of the antagonists have been chosen so that the differences between the combination of the two competitive antagonists and of the competitive and the functional antagonist are more emphasised than in Figs. a–b. It is clear that in (c) the low dose of atropine hardly contributes to the effect if combined with the higher dose of lachesine, while in case (d) the dose of isoprenaline equiactive with the dose of atropine in (c) gives a strong contribution to the effect if combined with lachesine; there then is nearly a summation in the antagonistic actions of the single compounds.

The differences between the actions of the competitive antagonist lachesine and of an antagonist such as isoprenaline are also manifested in the almost unrestricted shift in the dose-response curves for furtrethonium achieved by increasing doses of lachesine and the restricted shift with isoprenaline. Experiments with combinations of histamine, an antihistimine and a catecholamine such as isoprenaline give analogous results.

SEQUENTIAL BLOCKAGE

In the foregoing section, two types of combinations of blocking drugs are mentioned: the combination of two competitive antagonists and the

combination of a competitive and a functional antagonist. A special type of combination extensively discussed in the literature, especially on antibacterial chemotherapy, is the sequential blockage (Black, 1963). It can be defined as the blockage caused by a combination of two inhibitors that act on different receptors in a linear sequence of reactions (enzymes) (Black, 1963; Ariëns, 1964). Some of the most evident examples are the combination of a sulphonamide, which is a competitive antagonist of *p*-aminobenzoic acid, with a folic acid antagonist, such as aminopterin (see Fig. 9a). A combination related to the sequential blockage is

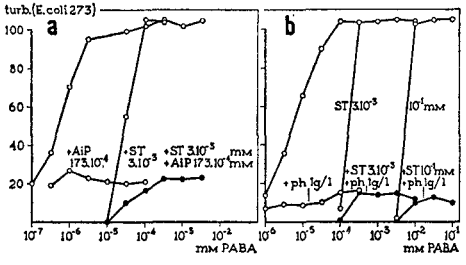


FIG. 9a, b. Log concentration-response curves for the growth of the PABA-deficient strain *E. coli* 273: (a) with PABA and the influence thereon of sulphathiazole (ST), of aminopterin (AiP) and of the combination of both antagonists; (b) with PABA and the influence thereon of sulphathiazole, of phenol and of the combination of both antagonists. Note that the effect of the combination of antagonists is practically the sum of the effects of the antagonists if applied singly.

obtained if in the last term in eqn 5, C is substituted for B, γ' for β' and K'_C for K'_B while $\alpha = 1$, $\beta = 0$ and $\gamma' = -1$. The eqn 10 then obtained represents the combination of an agonist A with a competitive antagonist B and a non-competitive antagonist C (Ariëns, 1964).

$$\frac{E_{ABC'}}{E_m} = \left[\frac{\alpha}{1 + (1 + [B]/K_B)\, K_A/[A]} + \frac{\beta}{1 + (1 + [A]/K_A)\, K_B/[B]}\right] \left[1 + \frac{\gamma'}{1 + K'_C/[C]}\right] \quad .. \quad .. \quad .. \quad (10)$$

Eqn 10 again like all the foregoing equations results from a simple application of the mass-action law to the various drug-receptor interactions involved. Fig. 10a represents an experimental example for a combination of drugs as just described. Dose-response curves are given for the spasmogen furtrethonium (a parasympathomimetic), tested in the presence of lachesine, a competitive antagonist (a neurotropic antispasmodic also called parasympatholytic); for furtrethonium tested in the presence of the musculotropic antispasmodic papaverine; and for furtrethonium in the presence of a combination of both antispasmodics mentioned. Fig. 9b gives another example. Here growth induced by the growth-factor *p*-aminobenzoic acid is inhibited by a combination of a competitive antagonist sulphathiazole and a non-competitive inhibitor phenol. Fig. 9b and 10a demonstrate that the combination of inhibitors which act independently, leads to a clear-cut potentiation, which may

be close to a summation of the inhibitive effects obtained with the drugs if applied singly.

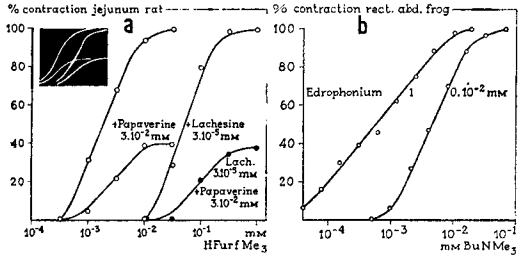


FIG. 10a, b. (a) Cumulative log concentration-response curves for the action of the parasympathomimetic furtrethonium ($HFurfMe_3$) and the influence thereon of the parasympatholytic lachesine, of the antispasmodic papaverine, and of the combination of both antagonists. Note the effect of the combination of lachesine and papaverine is practically the sum of the effects of the antagonists if applied singly. Compare the experimental curves with the set of theoretical curves (calculated from eqn 10) inset. (b) Cumulative log concentration-response curves for $BuNMe_3$ and the influence thereon of edrophonium. Note the sensitisation of the muscle to $BuNMe_3$ by edrophonium.

DESENSITISATION

Desensitisation in a general sense is a decrease in the response of a biological object to a drug as a result of the action of a previous dose of another drug or of a previous dose of the same drug. The various types of antagonism: competitive, non-competitive, and functional, described in earlier sections, can be seen as various forms of desensitisation as long as the antagonist is added first. In addition, interference with drug distribution and drug metabolism may result in a desensitisation. Take, for instance, the delay in resorption of a drug caused by a second drug, the inhibition of the bio-activation of a drug by a second one, or the enhancement of the excretion (Ariëns, 1964).

A special type of desensitisation is autodesensitisation, or tachyphylaxis (Champey & Gley, 1911). This is a decrease in the response to a dose of a drug given shortly after the effect of a previous equal dose of the same drug is abolished. After a number of doses the biological object may become fully unresponsive to the drug. Different types of tachyphylaxis can be distinguished depending on the causative mechanism. Some of the models for drug action mentioned in the preceding review imply the existence of tachyphylaxis.

(*a*) The "potential" theory says that a drug is active only as long as there is a net-flux of the drug into the cells or to the receptors, or in other words as long as there is a concentration gradient to the receptors. This implies that after removal of the drug from the extracellular phase the biological object will be desensitised to a sequential dose of the drug as long as there is an intracellular residue of the drug (Ariëns, 1964).

(*b*) The rate-theory postulates that there is a response only as long as there is a flux of drug molecules to the receptors, or, as long as there is

an association between drug molecules and receptors. After removal of the drug from the biophase, depending on the rate of dissociation, a proportion of the receptors will remain occupied for a time. During this period a desensitisation of the biological object to the drug given previously and also to related drugs will occur (Paton, 1961).

(*c*) The model for drugs acting indirectly, such as those sympathomimetics which are supposed to act by a liberation of endogenous catecholamines or liberators such as histamine liberators, requires a decrease in response after sequential dosage because the endogenous compound is depleted so the response is weakened (Axelrod, Gordon, Hertting, Kopin & Potter, 1962).

(*d*) The response induced in the biological object by a high dose of a drug may result in an exhaustion of the supply of energy or other factors necessary for the response to take place, and thus in a decrease in the responsiveness, which lasts until the system is regenerated.

In the cases (*a*), (*b*) and (*c*), the desensitisation will show a degree of specificity. It will occur only for drugs which have the same mechanism of action, i.e., drugs which intereact with the same receptors. In case (*d*) the desensitisation will occur for all drugs which make use of the effector system concerned. So, for instance, it will occur for all spasmogens where the contractile system in the biological object has reached exhaustion.

An experimental example of this type of desensitisation is given by Huidobro & Valette (1961). They described how, after high doses of acetylcholine or histamine, the isolated ileum of the guinea-pig becomes insensitive to spasmogens such as acetylcholine, histamine, barium chloride, 5-hydroxytryptamine, and also to coaxial electrical stimulation. After high doses of acetylcholine or histamine, organs, previously sensitised to specific antigens, did not respond with a contraction to these antigens.

(*e*) If the actions of sequential doses of a drug are studied *in vivo*, the response may be eliminated by the action of counter-regulating forces. The effect of a vasodepressor drug for instance may be abolished by an increase of vascular tone or heart frequency. Then the next dose may find most receptors still occupied, which may result in a desensitisation. Although many examples of the phenomenon of tachyphylaxis are given in literature (Furchgott, 1955; Blackman, 1961; Huidobro & Valette, 1961; Axelrod & others, 1962; Day & Rand, 1963), information on the mechanism is scarce. The types of autodesensitisation or tachyphylaxis described are reversible. Irreversible desensitisations also are possible although the term tachyphylaxis is not then applied.

If drug distribution and drug metabolism are also taken into account, further mechanism of autodesensitisation can be mentioned. This is, for example, where there is a decrease in sensitisation caused by an adaptive increase of degrading enzymes (Ariëns, 1964). However, relatively long times are necessary in these instances to induce the desensitisation which then lasts relatively longer; here, the term tachyphylaxis is best replaced by the term tolerance.

SENSITISATION

There are two essentially different types of sensitisation.

(1) *The sensitisation which results from repeated application of a drug or a preceding application of a related drug.* The time between applications may vary from several weeks to years. A minimum time, about 9 days, must elapse between the applications. The effects found after sensitisation differ from the effect caused by the first dose of the drug in that the reaction after sensitisation is hardly related to the type of drug used, but is closely related to the type of sensitisation. This sensitisation usually occurs because on first application, the drug acts as a hapten and this leads to the formation of antibodies against the hapten-protein complex formed. The second application of the same, or a chemically related, compound may then result in an allergic reaction.

(2) *The sensitisation which results in an increase in the response to a certain dose of a drug when applied after another, the sensitising drug.* Here, the effect after sensitisation is qualitatively of the same type as that before sensitisation, and specific for the type of drugs used. Usually, the time elapsing between the application of the compounds is restricted to minutes, hours, or, maximally, several days.

(*a*) The mechanism of the sensitisation may be based on changes in distribution or metabolism. Take, for instance, the increase in speed of absorption of drugs effected by hyaluronidase, the increased response to acetylcholine after inhibition of acetylcholinesterase, or the increase in the response to drugs as a result of the inhibition of renal excretion.

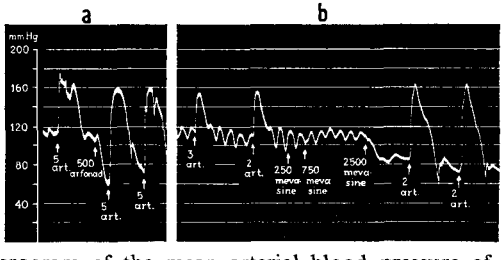


FIG. 11. Registerogram of the mean arterial blood pressure of the cat (2·5 kg) treated with noradrenaline (art.) and ganglionic blocking agents. Note that the changes induced in the blood pressure by noradrenaline (given intravenously) increase after intravenous injection of the ganglion agents trimethaphan camphorsulphonate (Arfonad) and mecamylamine (Mevasine). The vascular system is "sensitised" to noradrenaline by the ganglionic blocking agents.

(*b*) The sensitisation of the frog rectus muscle to acetylcholinomimetics such as butyltrimethylammonium ($BuNMe_3$) by acetylcholinesterase inhibitors such as neostigmine and edrophonium (Fig. 10b) should be mentioned here. Since $BuNMe_3$ is insensitive to acetylcholinesterase the sensitising action cannot be ascribed to the esterase-inhibiting properties of the sensitising drugs. Another example of a sensitisation is the sensitising action of sub-threshold doses of a stimulant drug, e.g. a spasmogen for related spasmogens (Uyldert, Tjiong & Ybema, 1955).

(*c*) An interesting example is the sensitisation of the frog rectus muscle for $BuNMe_3$ produced by an increase in the potassium concentration in the bath fluid. It works only if the potassium concentration is increased before the $BuNMe_3$ is added.

(*d*) A well-known type of sensitisation is the increase in response of a preparation to catecholamines after denervation or after depletion of the catecholamine stores by reserpine or cocaine. The decrease of the sympathetic tonus in the vascular system caused by ganglionic blocking agents also results in an increase of the response to catecholamines as is demonstrated in Fig. 11. Since, however, the maximal response to the catecholamines is hardly or not increased, it is arguable if there is a real sensitisation in this case.

(*e*) Blockade of counter-regulating processes by one drug may also result in an increase in response to a second one. The increase in the hypotensive response to β-sympathomimetic drugs such as phenyl t-butyl-arterenol after α-sympatholytic drugs are applied may serve as an example. The α-sympatholytic drug blocks the counter regulations based on vasoconstriction induced by endogenous noradrenaline and/or adrenaline on the α-receptors (Ariëns, Waelen, Sonneville & Simonis, 1963).

REVERSAL OF THE RESPONSE

In some instances, application of a drug may result in a reversal of the response to a second drug. Well-known examples are the change of the pressor response of adrenaline to a depressor response in the presence of α-sympatholytic drugs and the change of the depressor response of ethylarterenol to a pressor response in the presence of β-sympatholytic drugs (Ariëns & others, 1963). Reversals in response are common for drugs with a multiple action—drugs which induce effects on different types of receptors—if the effects induced on the various receptors have an opposite influence on the final effect studied. The α-sympathomimetic vasoconstriction induced on α-receptors and the β-sympathomimetic vasodilatation induced on β-receptors by adrenaline and ethylarterenol are examples. With adrenaline, the action on the α-receptors is dominant, with ethylarterenol the action on the β-receptors is dominant. The application of these drugs in the presence of compounds blocking the receptors on which the dominant action is induced, results in an unmasking of the opposite effect. Another well-known example of such a reversal is the change of the depressor action of acetylcholine on the parasympathetic division of the autonomic nervous system to a pressor response which originates from the ganglionic stimulant action in the sympathetic division, which is unmasked after parasympatholytics such as atropine.

The terms desensitisation, tachyphylaxis, sensitisation and reversal in action only indicate certain phenomena. They give no information on the specific processes of drug action involved. A closer analysis shows that various types of desensitisation, tachyphylaxis, etc., can be differentiated.

THE SLOPE OF THE DOSE-RESPONSE CURVES

It will have been seen there is a good agreement between the general characteristics of the sets of experimental dose-response curves and those of the theoretical curves which can be calculated from the equations given. However, there is a clear difference between theory and practice in the slope of the dose-response curves; in almost all cases the slope of the experimental curves is much steeper than that of the theoretical curves (eqn 1, preceding review). In exceptional cases in the literature, dose-response curves are described, in which the slope is virtually identical to that predicted by the theory (eqn 2, preceding review). In some cases this agreement is based on the plotting procedure of the dose-response curves. This is so, for instance, with the curves for acetylcholine tested on the frog rectus abdominis muscle described by van Maanen (1950) (Fig. 12a). This curve represents the dose-response relationship for a

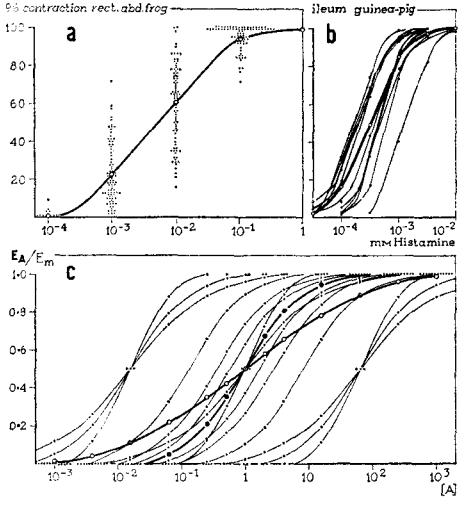


FIG. 12a, b. (a) The log dose-response curve for a group of frog rectus abdominis muscles to acetylcholine (ACh), obtained by calculating the mean response of the various muscles for different doses (van Maanen, 1950). Note that this curve is not the mean of the dose-response curves for the individual muscles. (b) Cumulative log concentration-response curves for histamine. The curves are obtained with pieces of gut of 12 different animals. Note that the slope of the mean curve for the group of organs is less steep than that of the curves for the individual organs. (c) Theoretical log concentration-response curves as calculated from eqn 2 (preceding review, p. 139) distributed symmetrically around the curve with an ED50 equal to 1. The heavy curve (○–○) represents the dose-response curve for the group of objects concerned. The steeper (bold) curve (●–●) represents the mean of the various individual dose-response curves. Note that the slope of the latter curve (●–●) is identical to the slope of the curves calculated from the theory. The slope of the other curve (○–○) is much less inclined.

group of organs; it is calculated on basis of the main response obtained with a number of doses on a number of biological objects. The slope of this curve is partially determined by the biological variation in the group of organs and is not identical with the slope of the dose-response curve for

a single object to which in fact the theoretical dose-response curve adheres (eqn 2, preceding review) (Perry, 1950; Gaddum, 1953). The curve obtained by van Maanen runs from the lower end of the dose-response curves for the objects which are highly sensitive, to the upper end of the dose-response curves for the objects with the lowest sensitivity. The larger the variation in the sensitivity of the biological objects, the larger will be the difference between the slope of the dose-response curve for a single object and the slope of the dose-response curve obtained with a group of biological objects. Fig. 12c demonstrates schematically the difference between the mean dose-response curve obtained with a group of organs and the mean of the dose-response curves obtained with a group of organs. Fig. 12b gives experimental evidence for the difference in the slope of the mean dose-response curve obtained with a group of organs and that of the curves obtained on single organs for the case of histamine tested *in vitro* on guinea-pig gut.

The difference between the slope of the experimental and theoretical dose-response curves does not seriously undermine the theory because there are various experimentally founded possibilities to interpret this difference such as the occurrence of threshold phenomena and the receptor reserve.

THRESHOLD PHENOMENA

Without going into detail, about the possible theoretical backgrounds of threshold phenomena such as the all-or-none response or mechanical factors (Ariëns & van Rossum, 1957b), experimental evidence will be given for thresholds in the dose-response curves for some types of drugs. A threshold implies that the effect, for instance the contraction of the muscular organ, only occurs after a certain concentration or dose of the drug is reached, with the consequence that the dose-response curves plotted on a linear scale will not pass through the origin (the zero point on the effect axis) but through some virtual point below. Fig. 13 gives

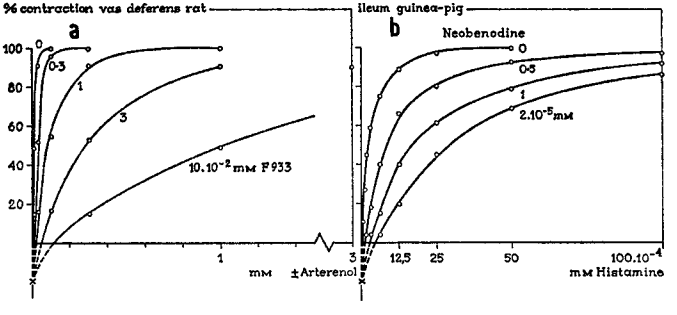


FIG. 13a, b. Cumulative concentration-response curves for the agonistic compounds noradrenaline (arterenol) and histamine and the influence thereon of various concentrations of the competitive antagonists benzodioxane (F933) and neobenodine respectively. Note how the curves intersect with the ordinate at a point below zero which indicates a threshold in the concentration-effect relation and gives the value for this threshold.

examples of dose-response curves which represent this phenomenon. Many other examples are known (Kirschner & Stone, 1951; Ariëns & Simonis, 1960; 1961). Because of the competitive relationship between the stimulant drugs noradrenaline and histamine and the antagonists benzodioxane and neobenodine (2-(*p*-methoxydiphenylmethoxy)-ethyldimethylamine), respectively, plotting the dose-response curves represented in Fig. 13 according to Lineweaver & Burk, would be expected to result in straight lines. As is shown in Fig. 14 this is not so. If, however,

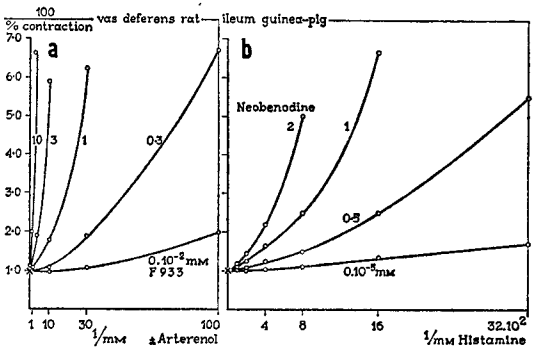


FIG. 14a, b. Cumulative concentration-response curves as represented in Fig. 13a, b, now plotted according to Lineweaver and Burk. Note that because of the competitive relation between agonist and antagonist the curves were expected to be straight.

a correction is made for the threshold according to the methods suggested by Kirschner & others (1951) the curves are straightened (see Fig. 15). These experimental results are strong evidence for a threshold in the dose-effect curves. The presence of a threshold implies that the experimental dose-response curves will differ in slope from the theoretical curves calculated from eqn 2 of the preceding review. The dose range over which the experimental curve is extended, is shortened on the side

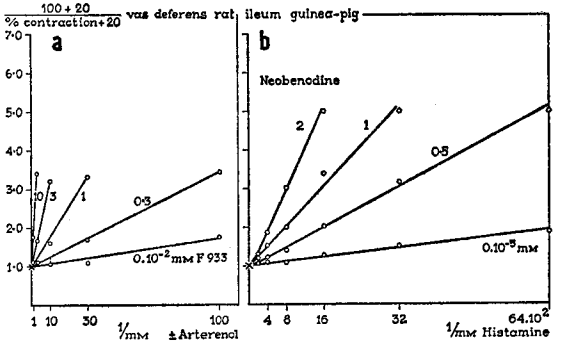


FIG. 15a, b. Cumulative concentration-response curves as represented in Figs 13a, b and 14a, b, plotted according to Lineweaver and Burk and now corrected for the threshold value as found in Fig. 13a, b. Note that after correction for the threshold value, straight lines are obtained.

of the lower concentrations and this curve will therefore be steeper than the theoretical curve which is based on the assumption of a proportionality between the fraction of the receptors occupied and the effect.

RECEPTOR RESERVE

As was emphasised by various investigators, the assumption that the effect is proportional to the fraction of receptors occupied, does not hold experimentally (Furchgott, 1954, 1955; Nickerson, 1956, 1957). In many cases a maximum effect, a 100% effect, is obtained after only a fraction of the receptors are occupied (Ariëns, van Rossum & Koopman, 1960; Ariëns, 1964). The existence of a receptor reserve can be demonstrated by making use of irreversible blocking agents of the specific receptors, such as the β-haloalkylamines of which dibenamine is an example.

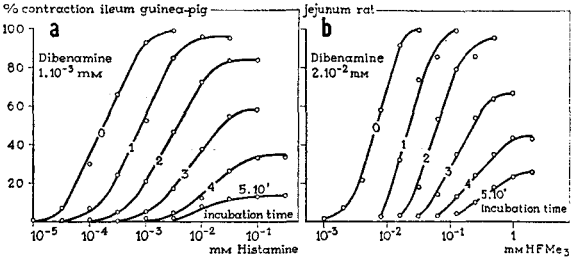


FIG. 16a, b. Cumulative log concentration-response curves for histamine (a) and $HFMe_3$ (b) after incubation of the organs with constant concentrations of dibenamine for various times. Incubation time n × 10 min. Note the shift which precedes a decline in the curves for histamine and $HFMe_3$, which indicates a receptor reserve.

Incubation of the biological object with an agent irreversibly blocking the specific receptors would be expected to lead to a decline in the dose-response curve. It nevertheless often leads to a parallel shift in the dose-response curves. This implies that after irreversible elimination

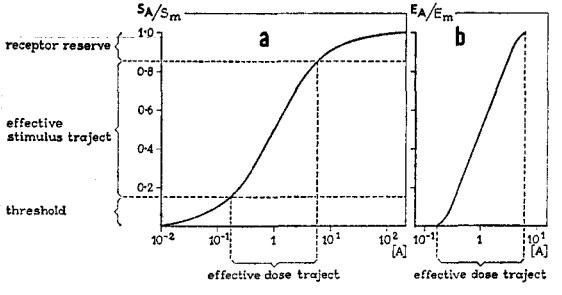


FIG. 17a, b. Theoretical curve representing the relation between the dose and the stimulus calculated from eqn 2 (preceding review, p. 139) in which S_A/S_m is substituted for E_A/E_m. (a) If a threshold and a receptor reserve as indicated are assumed—which implies no linear proportionality between stimulus and effect—a dose-effect curve as represented in (b) is obtained. Note the slope of this dose-effect curve is steeper than that of the dose-effect curve in the case where the effect is linearly proportional to the stimulus (no threshold and no receptor reserve). Then the dose-effect curve is identical to the dose-stimulus curve (a).

of a fraction of the receptors a 100% effect is still possible if higher doses of the stimulant drug are used. This means that there is a reserve in receptors. At the moment that the reserve is exhausted by the irreversible blocking agent, further elimination of receptors by this agent will lead to a decline in the slope of the dose-response curve and a decrease in the maximal effect obtained. The larger the shift in the curves that can be obtained with the irreversible blocking agent, the larger is the receptor reserve. Fig. 16a and b give experimental examples for the case of histamine and the parasympathomimetic $HFMe_3$. The existence of a receptor reserve implies that the experimental curve will be steeper than the theoretical dose-response curve calculated from eqn 2 of the preceding review. The dose range over which the experimental dose-response curve is extended is shortened on the side of the higher concentrations. Both the threshold phenomenon and the receptor reserve bring about an increase in the slope of the experimental dose-response curves, compared with that expected from theory (see Fig. 17).

RECEPTOR PROTECTION EXPERIMENTS

An interesting aspect of the irreversible blockade of specific receptors mentioned in the above section is the possibility of protecting the specific receptors against irreversible blockade by high doses of the agonist, related agonistic compounds, or reversible competitive antagonists. Their presence on the receptors will delay the irreversible blockade and thus result in a protection (Furchgott, 1955; Ariëns & others, 1960). This receptor protection will be specific, which means that a cross protection will occur for drugs which act on the same receptors. So, for instance

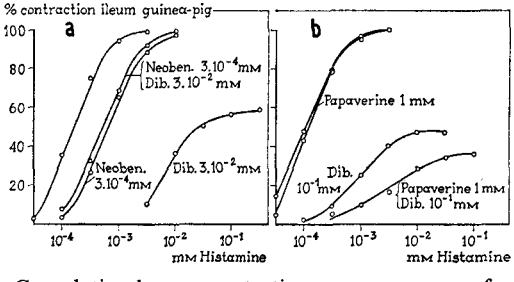


FIG. 18a, b. (a) Cumulative log concentration-response curves for the spasmogen histamine and the influence thereon of incubation of the organ with the irreversible blocking agent, dibenamine, for 10 min. and with dibenamine in the presence of the reversible blocking agent, the antihistamine neobenodine for 15 min. The reversible blocking agent was added 5 min. before dibenamine. Before adding the agonist after incubation, the organ was washed for 10 min. Note in the presence of the reversible blocking agent, a competitive antagonist, the organ is protected against the action of dibenamine. (b) Cumulative log concentration-response curves for the spasmogen histamine and the influence thereon of incubation of the organ with dibenamine for 10 min and with dibenamine in the presence of the reversible non-competitive antagonist papaverine for 15 min. Papaverine was added 5 min before dibenamine. Before adding the agonist after incubation, the organ was washed for 40 min. Note that, in contrast to the case represented in (a) the presence of the non-competitive antagonist (papaverine) does not protect the organ against the action of dibenamine.

the receptors for histamine can be protected by histamine and antihistamines (see Fig. 18a), but not by non-competitive agonists such as papaverine (see Fig. 18b) (Ariëns & Simonis, 1962a). Also here, there is an agreement between the experimental results and the theory as can be easily seen from the equations or the theoretical curves calculated on basis of them (Ariëns & others, 1960; Ariëns, 1964). The types of experiment described are by no means restricted to isolated organs such as the gut, or vas deferens. The principles of competitive and non-competitive interaction, reversible and irreversible binding hold true also in the field of microbiology, for example. The inhibition of growth of a *p*-aminobenzoic acid (PABA)-requiring strain of *Escherichia coli* 273 by specific antagonists of PABA, such as sulphathiazole and 2-methyl-PABA, may serve as an example. Fig. 19a and 19b represent the inhibition of growth if the growth factor, PABA, is added at the same time as the inhibitors. The curves argue for a competitive relationship between growth factor and inhibitor.

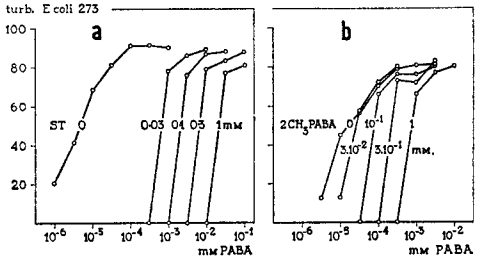


FIG. 19a, b. Log concentration-response curves for the growth (turbidity) of the *p*-aminobenzoic acid (PABA)-requiring strain *E. coli* 273 and the influence thereon of various concentrations of the antimetabolites sulphathiazole (ST) (a) and 2-CH_3-PABA (b). Metabolite and antimetabolite are added simultaneously. Note the sets of curves are characteristic for the competitive relation between PABA and the antimetabolites.

The growth factor, PABA, is assumed to serve as a precursor for folic acid and folinic acid respectively, which in its turn serves as a coenzyme F in an enzyme that plays a rôle in the transfer of one-carbon units. If the chemical structures of PABA, 2-methyl-PABA and sulphathiazole are compared it will be clear that 2-methyl-PABA could possibly substitute for PABA in the folic acid molecule, while this seems unlikely for sulphathiazole.

The incorporation of 2-methyl-PABA in folic acid and finally in coenzyme F means that this inhibitor is practically irreversibly bound. If *E. coli* 273 in the logarithmic phase of its growth is incubated with 2-methyl-PABA and sulphathiazole respectively and, after incubation, the growth factor PABA is added, the inhibition of growth by sulphathiazole can be overcome easily by the PABA while in the cultures incubated with 2-methyl-PABA no growth or only some growth after a long delay can be obtained. These experiments suggest that 2-methyl-PABA is assimilated to give an afunctional folic acid and finally an afunctional coenzyme F—while the sulphonamide competes with PABA in a strictly reversible way, possibly at the entrance of the metabolic channel for PABA.

If PABA and 2-methyl-PABA are applied simultaneously, PABA, if applied in sufficiently high concentrations, may protect the bacteria against the irreversible incorporation of 2-methyl-PABA. This is demonstrated in Fig. 20a and b (Ariëns & Simonis, 1962; Ariëns, 1964).

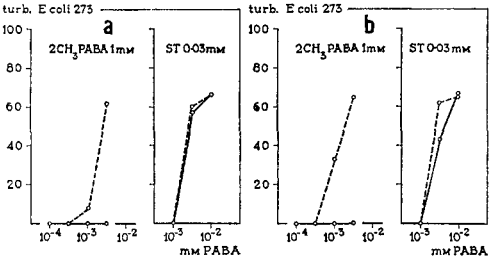


FIG. 20a, b. Log concentration-response curves for PABA as a growth factor for *E. Coli* 273. Curves – – – –: metabolite and antimetabolite are applied simultaneously. Curves ———: the same combinations of metabolite and antimetabolite, but now the metabolite is added after incubation with the antimetabolites for 15 hr (a) and for 30 hr (b). Note incubation with sulphathiazole does not change the results obtained. After incubation with 2-CH_3-PABA no further growth is obtained with PABA.

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