STUDIES ON THE MODE OF ANTAGONISM BETWEEN ADRENERGIC $\beta$-MIMETICS AND $\beta$-BLOCKING AGENTS (III)
FUNCTIONAL ANTAGONISM BETWEEN $\beta$-MIMETICS AND SPASMOGENS

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Abstract—New equations which can explain the following characteristic phenomena in the functional antagonism between isoproterenol (ISO) and spasmogens in guinea-pig trachea are proposed: (1) The amplitude of relaxation of the muscle induced by ISO varied depending on the concentration of a spasmogen used (histamine or carbachol). (2) The dose-response curves for the relaxation by ISO shifted to the right in a parallel manner as the concentration of the spasmogen increased, and became stationary at higher concentrations of the spasmogen. (3) The slope of the dose-response curve became steeper with increasing concentrations of the spasmogen. When the saturable uptake process of ISO was taken into consideration, a satisfactory parallel was seen between the theoretical dose-response curves and the present experimental results.

In order to increase the amplitude of sympathomimetic-induced relaxation of a guinea pig tracheal chain, the tracheal preparation is usually contracted with a certain spasmogen prior to the addition of the sympathomimetic amine. The spasmogen (histamine or carbachol) used in this case not only increases the amplitude of the response, but also may act as a functional antagonist and shift the dose-response curve of the sympathomimetics to the right (1). The $pD_2$ in this case must be determined from the equation of functional antagonism.

The model of functional antagonism was introduced by Ariëns et al. (1) who defined functional antagonism as follows: Two drugs, A and B, interact with different receptors, $R_I$ and $R_{II}$, while they produce their effect by means of a common effector $E$ in such a way that their contributions to the effect are opposite. This was represented by Eq. 1:

$$E_{m} = \frac{E_{IA}}{E_{m}} + \frac{E_{IB}}{E_{m}}$$

where $E_{IA}$ and $E_{IB}$ are the individual effects of A and B induced on the receptor system $R_I$ and $R_{II}$, respectively, and $E_m$ is the maximum effect possible with the effector system concerned. In the presence of a certain concentration of the functional antagonist [B] the effect induced by the agonist A is reduced by a constant, the term $E_{IB}/E_m$ of Eq. 1. However, Van den Brink pointed out that the over-all picture of experimental functional antagonism (isoproterenol-methacoline on calf trachea) was quite different from that calculated with Eq. 1, and postulated a new model of functional interaction allowing the concept of receptor
reserve (2). In this paper, another possible explanation is presented, and equations that can explain the experimental dose-response curve of functional antagonism between an adrenergic stimulant and a spasmogen, are introduced.

**MATERIALS AND METHODS**

*Theoretical considerations*

Increase in contraction (tone) by pretreatment with a spasmogen brings about an increase in amplitude of the relaxation of tracheal muscle by adrenergic \( \beta \) stimulants. On the other hand, \( \beta \) stimulants can relax the tracheal muscle even when it is not pretreated with the spasmogen. If one takes this relaxation as standard and the amplitude of relaxation is increased \( \delta \)-fold by pretreatment with a spasmogen, the amplitude of relaxation would be represented as follows:

\[
y = a \cdot S \cdot (1 + \delta)
\]

where \( y \) is the amplitude of relaxation (response), \( S \) is the stimulus, and \( a \) is the proportional constant. Since \( \delta \) increases with increasing concentrations of spasmogens, \( \delta \) is represented by Eq. 3:

\[
\delta = \frac{\gamma}{1 + \frac{K_F}{[F]}}
\]

where \( \gamma \) is the maximum effect, \( K_F \) is the dissociation constant of the spasmogen (or functional antagonist)-receptor complex, and \([F]\) is the concentration of the functional antagonist. Stimulus is reduced by functional antagonism (1) and this could be represented as follows:

\[
S = \frac{\alpha}{1 + \frac{K_A}{[A_h]}} - \frac{\beta}{1 + \frac{K_F}{[F]}}
\]

where \( \alpha \) is the maximum stimulus produced by the interaction of an agonist with the receptor, \( \beta \) is the maximum stimulus produced by a functional antagonist, \([A_h]\) is the concentration of the agonist in the region of the receptor, and \( K_A \) is the dissociation constant of the agonist-receptor complex. \([F]\) and \( K_F \) have the same meanings as in Eq. 3. The first term in Eq. 4 is the stimulus produced by the agonist at the concentration \([A_h]\) and the second term is that produced by the functional antagonist at the concentration \([F]\). Combining Eqs. 2, 3 and 4 gives Eq. 5:

\[
y = a \cdot \left( \frac{\alpha}{1 + \frac{K_A}{[A_h]}} - \frac{\beta}{1 + \frac{K_F}{[F]}} \right) \left( 1 + \frac{\gamma}{1 + \frac{K_F}{[F]}} \right)
\]

The maximum response in the presence of a certain concentration of the functional antagonist is represented by Eq. 6:
Dividing Eq. 5 by Eq. 6 gives Eq. 7, in which the response in the presence of both the agonist \([A_b]\) and the functional antagonist \([F]\) can be represented as a fraction of the maximum response.

\[
y = \frac{\alpha}{1 + \frac{K_A}{[A_b]} + \frac{\beta}{[F]}}
\]

\[
y_m = \frac{\alpha}{1 + \frac{\beta}{[F]}}
\]

If one takes into account the saturable uptake of an agonist, the relation between the concentration of the agonist in the region of the receptor, \([A_b]\), and the concentration of the agonist in the organ bath, \([A_a]\), is represented by Eq. 8 (3):

\[
\frac{[A_b]}{[A_a]} = \frac{[A_b] + K_{AU}}{[A_b] + K_{AU} + \frac{V_m}{k}}
\]

where \(K_{AU}\) is the Michaelis-Menten constant of the uptake process of the agonist, \(V_m\) is the maximum velocity of the uptake, and \(k\) is the diffusion constant of the agonist between the external solution and the region of the receptor. Eq. 8 can be transformed to Eq. 9:

\[
[A_a] = \frac{[A_b] - K_{AU} + \frac{V_m}{k} \sqrt{\left(\frac{K_{AU}}{k} + \frac{V_m}{k} [A_a]\right)^2 + 4 K_{AU} [A_a]}}{2}
\]

From Eqs. 7 and 9, the response, \(y\), can be calculated for a value of \([A_a]\).

When a competitive antagonist is present, the dose-response curve may be explained by Eq. 10.

\[
y = \frac{\alpha}{1 + \frac{K_A}{[A_b]} + \frac{\beta}{[B]}}
\]

\[
y_m = \frac{\alpha}{1 + \frac{\beta}{[F]}}
\]

where \([B]\) is the concentration of the competitive antagonist in the region of the receptor and \(K_B\) is the dissociation constant of the competitive antagonist-receptor complex.

The potency of a drug is often expressed as the concentration of the agonist which produces a half maximal effect. When a competitive antagonist is absent, this is expressed by Eq. 11.
This is rearranged to Eq. 12.

\[ [A_b]' = \phi \cdot K_A \]  (12)

When a competitive antagonist is present, this may be represented by Eq. 13.

\[ [A_b]' = \phi \cdot K_A \cdot \left( \frac{K_B}{[B]} - 1 \right) \]  (13)

In Eqs. 12 and 13, \( \phi \) is defined by Eq. 14, and \([A_b]’\) is the concentration of the agonist which produces a half-maximal response, i.e., the apparent dissociation constant.

\[ \phi = \frac{\alpha}{1 + \frac{\beta}{K_F}} \]  (14)

Eqs. 12 and 13 indicate that the apparent dissociation constant is increased \( \phi \)-fold by functional antagonism.

**Experimental**

Pairs of guinea-pig tracheal preparations (5) were suspended in an organ bath containing 20 ml of Tyrode's solution at 37°C, bubbled with air. Mechanical responses were recorded isotonically on a kymograph.

**Drugs**

\((\pm)\)-Isoproterenol HCl (ISO) (Boehringer Sohn) was dissolved in a solution containing 0.8% NaCl and 0.05% NaHSO₃. Histamine 2HCl (HA) (Tokyo Kasei) and carbachol HCl (Cch) (Tokyo Kasei) were dissolved in water. Cumulative dose-response curves of ISO were obtained by increasing stepwise the concentration of ISO by a factor of about 3 while the previous dose remained in contact with the tissue. All other solutions were added in a volume of 0.2 ml.

**Contraction of guinea-pig tracheal preparations by spasmogens and effects of dibenamine**

After the tracheal preparations were allowed to equilibrate for about 2 hr, cumulative dose-response curves of the contraction by the two spasmogens, histamine and carbachol, were constructed by increasing the concentrations in the bath by a factor of about 3 while the previous dose remained in contact with the tissue. After the contraction by one spasmogen reached a maximum, the other spasmogen was added to examine whether or not the pre-
The preparation was then washed with Tyrode's solution at intervals of about 5 min for 30 min, treated with dibenamine (30 µg/ml) for 30 min and washed again with Tyrode's solution at intervals of about 5 min for another 30 min. A second cumulative dose-response curve for the spasmogen was then obtained to determine the effect of the dibenamine treatment.

**Effect of spasmogens (histamine and carbachol) on the dose-response curves of ISO**

The preparations were allowed to equilibrate for about 2 hr. After contraction by a spasmogen, dose-response curves of the relaxation by ISO were obtained by increasing stepwise the concentration of ISO in the same manner as outlined above. Each addition was made only after the effect of the previous addition had reached a maximum and remained constant.

**RESULTS**

The dose-response curves of the contraction of a guinea-pig tracheal chain by carbachol (Cch) and histamine (HA) and the effect of dibenamine on these curves are shown in Fig. 1. The pD₂ of Cch and HA was 6.84±0.06 (n=8) and 5.27±0.05 (n=8), respectively. After the contraction by HA had reached the maximum, Cch contracted the preparation further. On the other hand, after the contraction by Cch reached the maximum, HA no longer contracted the preparation. The maximum contraction induced by HA was about 70% of that induced by Cch. Pretreatment with dibenamine inhibited exclusively the contraction by HA, although the contraction caused by lower concentrations of Cch was also inhibited by the dibenamine treatment as shown in Fig. 1.

The dose-response curves of the relaxation of a guinea-pig tracheal chain by ISO in the presence of HA are shown in Fig. 2A. The amplitude of relaxation of the muscle was increased about 2.4-fold by pretreatment with HA (10 µg/ml), whereas the apparent pD₂ of ISO defined as the concentration of the agonist which produces a half maximal response decreased from 8.30 to 7.73. Fig. 2B shows the corresponding theoretical dose-response curves obtained with Eq. 5 and Eq. 9, using the following parameters: α = 1.0, β = 0.65, γ = 8.0, Kₐ = 10⁻⁹.₁₈ M, Kᵢ = 10⁻⁵.₃₇ M, \( \frac{V_m}{k \cdot K_{AU}} = 7 \), [F] = 10⁻⁴.₂₇ M, a = 1, and Kₐₑ = 10⁻⁷.₀ M. The γ and α values were chosen so that the calculated maximum amplitude paralleled the one observed. These theoretical curves fitted the experimentally obtained dose-response
FIG. 2.  
A Dose-response curves for the relaxation of guinea-pig tracheal chain by ISO.  
–○– pretreated with HA (10⁻⁶ g/ml).  –●– without spasmogen.  
B Theoretical curves obtained from Eqs. 5 and 9.  The solid lines are theoretical curves obtained 
by using the following parameters:  \( \alpha = 1.0, \beta = 0.65, \sigma = 1.0, \gamma = 8.0, K_A = 10^{-9.15} M, \)
\( K_F = 10^{-9.27} M, \)  \( V_m \) = P = 7.  \([F] = 10^{-9.27} M \)  
and  \( K_{AU} = 10^{-7.6} M. \)  
The broken lines are those obtained by using the following parameters:  \( \alpha = 1.0, \)
\( \sigma = 1.0, \beta = \gamma = 0.00, K_A = 10^{-9.18} M, P = 7, \)  
and  \( K_{AU} = 10^{-7.6} M. \)

FIG. 3.  
A The rightward shift of the dose-response curve for the relaxation of a guinea-pig tracheal chains by ISO in the presence of increasing concentrations of histamine.  
B Theoretical dose-response curves obtained by calculation with the following 
parameters:  \( \alpha = 1, \beta = 0.65, \)  \( V_m = P = 7, \)  
\( K_{AU} = 10^{-7.6} M, K_A = 10^{-9.15} M, \)  
and  \( K_F = 10^{-9.27} M. \)  \([F] \) is increased from 10⁻⁶.275 M to 10⁻³.275 M.

FIG. 4.  
The rightward shift of the dose-response curve for the relaxation of a guinea-pig tracheal chain by ISO in the presence of increasing concentrations of carbachol.  
B Theoretical dose-response curves obtained by calculation with the following 
parameters:  \( \alpha = 1, \beta = 0.97, P = 7, K_{AU} = 10^{-7.6} M, K_A = 10^{-9.18} M \)  
and  \( K_F = 10^{-9.54} M. \)  \([F] \) is increased from 10⁻⁷.755 M to 10⁻⁴.755 M.
curves shown in Fig. 2A. Fig. 3A shows that the dose-response curve of ISO shifts to the right as the concentrations of histamine increases. The corresponding theoretical curves obtained with Eqs. 7 and 9 using the following parameters are shown in Fig. 3B: \( \alpha = 1 \), \( \beta = 0.65 \), \( \frac{V_m}{k \cdot K_{AU}} = 7 \), \( K_{AU} = 10^{-7.00} \) M, \( K_A = 10^{-8.2} \) M and \( K_F = 10^{-5.27} \) M. Fig. 4A shows the rightward shift of the dose-response curve of ISO with increasing concentrations of Cch. The corresponding theoretical curves are shown in Fig. 4B. These curves were calculated with Eqs. 7 and 9 using the following parameters: \( \alpha = 1 \), \( \beta = 0.97 \), \( \frac{V_m}{k \cdot K_{AU}} = 7 \), \( K_{AU} = 10^{-7.00} \) M, \( K_A = 10^{-9.2} \) M and \( K_F = 10^{-6.84} \) M. The values of \( \beta \), 0.65 and 0.97 for HA and Cch, respectively, correspond to the result that the relative magnitude of the maximum contraction induced by HA was about 70% of that induced by Cch as shown in Fig. 1. The values of \( P \) and \( K_F \) for ISO were chosen from the experimental results in a preceding paper (4). It is theoretically predicted that the activity of an agonist will be potentiated \( (P+1) \)-fold when the uptake process is inhibited. Since the activity of ISO was potentiated about 8-fold by inhibiting its uptake process with dibenamine (4), the value of \( P \) was estimated to be 7 for ISO. The values of \( K_A \) and \( K_{AU} \) for ISO were also quoted from a preceding paper (4). The values of \( K_F \) for the curves in Figs. 3B and 4B correspond to the \( pD_2 \) values of HA and Cch, respectively (Fig. 1). Thus, the values of all parameters \( (\alpha, \beta, P, K_A, K_F \) and \( K_{AU} \) but except \( r \) \) for theoretical calculations were estimated from the experimental results. Agreement between the results of theoretical calculation and experimental results was quite satisfactory as shown in Figs. 2-4. The apparent \( pD_2 \) of ISO in relaxation of guinea-pig trachea decreased with increasing concentrations of the spasmogen. These values are summarized in the table with theoretically estimated apparent \( pD_2 \) and both the observed and calculated maximal amplitudes of relaxation.

**DISCUSSION**

In order to increase the amplitude of relaxation of a tracheal chain by sympathomimetics, the preparation is usually contracted with a certain spasmogen before the relaxation is induced. However, these agents also act as functional antagonists and response curves shift to the right. As shown in Figs. 3 and 4, the magnitude of this shift is dependent on the concentration of the spasmogen used. The slope of the curve became steeper with increasing concentration of the spasmogen, and the shift became minimal at higher concentrations of the functional antagonist. Eq. 1 that has been used for functional antagonism cannot adequately explain such phenomena. Van den Brink proposed a new model of functional antagonism allowing the concept of receptor reserve (2). Both the parallel shift of the dose-response curve to the right in the presence of a functional antagonist and the slowing rate of the shift with increasing concentrations of the antagonist can indeed be explained by this model. However, agreement between the experimental results and the theoretical curves did not appear to be quantitatively satisfactory.

In this paper simple equations of functional antagonism (Eqs. 5 and 7) are proposed.
By these equations the following characteristic phenomena can be explained. (1) The maximal amplitude of relaxation varies with the concentration of a spasmogen used. There is an optimum concentration of each spasmogen that leads to the maximum relaxation by \( \beta \) stimulants. (2) The dose-response curve is shifted to the right in a parallel manner, and becomes stationary at higher concentrations of the functional antagonist. (3) The slope of the curve becomes steeper with increasing concentrations of the spasmogen. In Eqs. 5 and 7, \( \alpha \) and \( \beta \) signify the relative strengths of the maximum stimulus. In the ISO-Cch functional antagonism, agreement between the theoretical and experimental curves were quite satisfactory when \( \alpha = 1 \) and \( \beta = 0.97 \) (Fig. 4), whereas in the ISO-HA functional antagonism agreement was satisfactory when \( \alpha = 1 \) and \( \beta = 0.65 \) (Fig. 3). It is interesting that the ratio of the \( \beta \) values for Cch and HA (0.97 : 0.65) is in accordance with the ratio of the magnitudes of the maximum contraction elicited by Cch and HA (1 : 0.7). The theoretical estimates were in good agreement with the experimental results as shown in Figs. 2-4, and the values of all the parameters except \( \gamma \) and \( \alpha \) (these values are arbitrarily set) in the equations were consistent with the other experimental results. Thus, Eq. 5 and Eq. 7 may be appropriate, and give useful descriptions, for functional antagonism.

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