STUDIES ON THE MODE OF ANTAGONISM BETWEEN ADRENERGIC β-MIMETICS AND β-BLOCKING AGENTS (IV)

INFLUENCE OF FUNCTIONAL ANTAGONISM BY SPASMOGENS

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Abstract—A new theory is presented to describe the effect of functional antagonism on the competitive antagonism between adrenergic β-mimetics and β-blockers. According to this theory the shape of the log (dose ratio-1) vs. -log [B] curve and the apparent pA2 value in competitive antagonism should be affected by functional antagonism when the agonist is taken up by the saturable uptake process, and this was experimentally confirmed. The competitive antagonism between isoproterenol (ISO) and propranolol (Prop) was influenced by the functional antagonism between ISO and spasogens (histamine and carbachol). The log (dose ratio-1) vs. -log [B] curve of ISO-Prop competitive antagonism was shifted variously depending on the concentration of a spasogen used. Theoretical predictions and experimental results were in good parallel.

When relaxation of a guinea-pig tracheal preparation by sympathomimetics is measured, the preparation is usually contracted beforehand by a certain spasogen in order to increase the amplitude of the relaxational response. The spasogen used in this case not only increases the amplitude of the response, but also acts as a functional antagonist, and shifts the dose response curve of sympathomimetics to the right (1). According to Schild’s theory (2), competitive antagonism should not be affected by functional antagonism. This paper presents a new theory with experimental evidence to show that functional antagonism influences the shape of the curve of competitive antagonism when a saturable uptake process for the agonist exists.

MATERIALS AND METHODS

Theoretical consideration

The pA2 has so far been determined from Eq. 1 (2).

\[
\frac{[A]}{[A]_o} = \frac{[B]}{K_B} + 1
\]  

(1)

In a preceding paper (3), the dose ratio \[ \frac{[A]}{[A]_o} \] in the competitive antagonism involving the saturable uptake process of the agonist was shown to be represented by Eq. 2, instead of Eq. 1.

\[
X = \left( \frac{[B]}{K_B} + 1 \right) \left( \frac{U - 1}{U + P - 1} \right) \left( 1 + \frac{P}{1 - U \left( \frac{[B]}{K_B} + 1 \right) + \frac{[B]}{K_{RU}}} \right)
\]  

(2)
where \( X \) is the ratio of concentrations of the agonist in the bathing solution giving equal response in the presence and absence of an antagonist, \( K_F \) is the dissociation constant of the antagonist-receptor complex, \( K_{RU} \) is the dissociation constant of the antagonist-uptake site complex, \( U \) is the ratio of the dissociation constant of the agonist-receptor complex to the Michaelis-Menten constant of the uptake process of the agonist (i.e., a measure of easiness of the agonist uptake). \( P \) is an estimate of the degree of potentiation of the agonist when the uptake process is blocked, and \( [B] \) is the concentration of the antagonist in the region of receptors, and may be in equilibrium with the concentration in the organ bath.

As described previously (1), functional antagonism shifts the dose-response curve to the right. When a competitive antagonist is absent, the concentration of the agonist \([A_b]_o\) that produces a half maximal effect is increased \( \phi \)-fold by functional antagonism (1):

\[
[A_b]_o = \phi \cdot K_A
\]  

(3)

When a competitive antagonist is present, the corresponding concentration \([A_b]_o\) is represented by Eq. 4 (1):

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

(4)

In Eqs. 3 and 4, \( \phi \) is defined as follows:

\[
\phi = \frac{\alpha \cdot \beta}{1 + \frac{K_F}{[F]}}
\]  

(5)

where \( \alpha \) and \( \beta \) are the maximum effects produced by the agonist and the functional antagonist, respectively, \( K_F \) is the dissociation constant of the functional antagonist-receptor complex, and \([F] \) is the concentration of the functional antagonist. When there is saturable uptake of an agonist, the relationship between the concentration of the agonist in the region of the receptor, \([A_b]_o\), and the concentration of the agonist in the organ bath, \([A_a]_o\), is represented by Eq. 6 (4).

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

(6)

When a competitive antagonist is present, the same relation is represented by Eqs. 7 (3):

\[
\frac{[A_b]}{[A_a]} = \frac{[A_b] + K_{AU} \left( \frac{[B]}{K_{BU}} + 1 \right)}{[A_b] + K_{AU} \left( \frac{[B]}{K_{BU}} + 1 \right) + \frac{V_m}{k}}
\]  

(7)

Combining Eqs. 3 and 6 gives:
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\[
[A_a]_o = \frac{(\phi \cdot K_A)^2 \cdot \left( K_{AU} + \frac{V_m}{k} \right) \cdot \phi \cdot K_A}{\phi \cdot K_A + K_{AU}} \tag{8}
\]

Combining Eqs. 4 and 7 gives:

\[
[A_a]_o = \frac{(\phi \cdot K_A)^2 \cdot \left( K_B + 1 \right) \cdot \phi K_A K_{AU} \cdot \left( \frac{[B]}{K_{BU} + 1} + 1 \right) + \phi K_A \cdot \frac{V_m}{k} \cdot \left( K_B + 1 \right)}{\phi K_A \left( \frac{[B]}{K_B + 1} + K_{AU} \left( \frac{[B]}{K_{BU} + 1} + 1 \right) \right)} \tag{9}
\]

In Eqs. 8 and 9, \([A_a]_o\) and \([A_a]\) represent the doses of the agonist which produce a half maximal response in the absence and presence of a competitive antagonist, respectively.

Combining Eqs. 8 and 9 gives the dose ratio \(X\):

\[
X = \left( \frac{[B]}{K_B + 1} \right) \left( \frac{\phi U + 1}{\phi U + P - 1} \right) \left\{ 1 + \frac{P}{1 + \phi U \left( \frac{[B]}{K_B + 1} \right) + \frac{[B]}{K_{BU}}} \right\} \tag{10}
\]

where \(X\), \(U\) and \(P\) are abbreviations of \(\frac{[A_a]_o}{[A_a]}\), \(\frac{K_A}{K_{AU}}\) and \(\frac{V_m}{k \cdot K_{AU}}\), respectively (3).

It is theoretically predicted that the shape of the \(\log (X - 1)\) vs. \(-\log [B]\) curve of competitive antagonism changes with the \(U\) value of an agonist (3). It has been shown that, when two agonists (ISO and TMQ) have different \(U\) values, the shapes of the curves for competitive antagonist are different (3).

As indicated in Eq. 10, \(U\) is multiplied by \(\phi\). This means that \(U\) in Eq. 2 is increased \(\phi\)-fold by functional antagonism. The \(\phi\) value varies depending on the concentration of a functional antagonist as shown in Eq. 5. The shape of the curve for competitive antagonism would vary with the \(\phi \cdot U\) value which depends on the concentration of a functional antagonist. If an agonist is not continuously removed from the receptor region, competitive antagonism is represented by Eq. 1. In this case, functional antagonism does not influence competitive antagonism. Conversely, one could examine whether or not an agonist is removed by the saturable uptake process by studying the effect of functional antagonism on the curve of competitive antagonism.

In summary, the shape of the \(\log (X - 1)\) vs. \(-\log [B]\) curve of competitive antagonism varies depending on the concentration of a functional antagonist when an agonist is taken up by the saturable uptake process and in this case the dose ratio \(X\) for a half maximal response in the presence and absence of competitive antagonist is represented by Eq. 10.

**Experimental**

Mechanical responses of isolated tracheal muscle preparations of the guinea-pig were recorded by means of isotonic lever on a kymograph. (–)-Isoproterenol HCl (ISO) (Boehringer Sohn) was dissolved in a solution containing 0.8% NaCl and 0.05% NaHSO₄, (±)-propranolol HCl (Prop). (I. C. I.), histamine 2HCl (HA) (Tokyo Kasei) and carbachol
HCl (Chh) (Tokyo Kasei) were dissolved in water.

**Effects of spasmogens on ISO-Prop antagonism with guinea-pig tracheal chain preparations**

Pairs of tracheal chain preparations (5) were suspended in an organ bath containing 20 ml of Tyrode's solution at 37°C, bubbled with air. The preparations were allowed to equilibrate for about 2 hr. After contraction had reached a maximum with a certain dose of a spasmogen and remained constant, a cumulative dose-response curve of ISO was obtained by increasing the concentration by a factor of about 3 while the previous dose remained in contact with the tissue. Each addition of ISO was made only after the effect of the preceding concentration reached maximum. The preparation was washed with Tyrode's solution at intervals of about 10 min for 30 min, Prop was added to the organ bath 30 min before the second contraction by the spasmogen, and a second series of cumulative additions of ISO were made to obtain another dose response curve of ISO in the presence of Prop. These steps were repeated with various concentrations of Prop.

**RESULTS**

The curve in the presence of HA was markedly different from the curve in the absence of a spasmogen even if the other conditions were the same (Fig. 1).

The effect of Chh on the competitive antagonism between ISO and Prop is shown in Fig. 2. The log (X−1) vs. −log [B] curve shifted to the left and downward as the concentration of Chh was increased from 10^{-7.76} M to 10^{-5.70} M and then it shifted in the opposite direction with a further increase in the concentration of Chh to 10^{-4.76} M.

As shown in Figs. 3 and 4, the theoretical curve of log (X−1) vs. −log [B] is depressed as the ϕ·U value increases up to 1, and then it is elevated as the ϕ·U value increase from

![Fig. 1. The log (X−1) vs. −log [B] curves of ISO-Prop antagonism in the absence of spasmogens (○) and in the presence of 10^{-4.275} M of HA (●). The thick lines are theoretical curves for U=0.006 ( ) and U=0.65 (——). All the other parameters were set as follows; P=7, −log K_B=9.2 and −log K_{IU}-5. The thin dotted line is the theoretical curve for P=U=0 (no uptake).](image1)

![Fig. 2. The log (X−1) vs. −log [B] curves of ISO-Prop antagonism in the presence of carbachol of 10^{-6.76} M (○), 10^{-5.76} M (●), and 10^{-4.76} M (●). The thick lines are theoretical curves for U=0.03 ( ), U=0.2 (——) and U=5 (———). All the other parameters were set as follows; P=7, −log K_B=9.2, −log K_{IU}=5. The thin dotted line is the theoretical curve for P=U=0 (no uptake).](image2)
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Fig. 3. A series of theoretical curves obtained by Eq. 10, when $U$ varied from 0.001 to 1. The $U$ values are shown on the corresponding curves. For all curves, $P=7$, $-\log K_B=5$ and $-\log K_U=9.2$.

Fig. 4. A series of the theoretical curves obtained by Eq. 10 when $U$ varied from 1 to 50. The $U$ values are shown on the corresponding curves. For all curves, $P=7$, $-\log K_B=5$ and $-\log K_U=9.2$.

1 to $\infty$. Increasing the concentration of Cch changes the $\phi$ value as defined by Eq. 5. When $\alpha$ was 1 and $\beta$ was 0.97 (1), the $\phi$ values calculated with Eq. 5 were 3.35, 18.78 and 52.1 at $10^{-6.76}$, $10^{-5.76}$ and $10^{-4.76}$ molar concentrations of Cch, respectively. From the shape of the experimental curve without a spasmogen shown in Fig. 2, the $U$ value of ISO was estimated to be about 0.003. Then, the calculated $\phi \cdot U$ values become 0.01, 0.1 and 0.3 at $10^{-6.76}$, $10^{-5.76}$, and $10^{-4.76}$ molar concentration of Cch, respectively. At the corresponding concentrations of Cch, the $\phi \cdot U$ values were found to be about 0.02, 0.1 and 0.3, respectively, from the curves in Fig. 2. At $10^{-4.275}$ molar concentration of HA the $\phi$ value was 3.88, and therefore $\phi \cdot U$ value became about 0.01, while the $\phi \cdot U$ value in this case was observed to be about 0.05 from the curves in Fig. 1. Although the calculated $\phi \cdot U$ values were not in perfect agreement with the observed values, the experimental results (Figs. 1 and 2) were essentially in good agreement with the theoretical curves (Figs. 3 and 4).

DISCUSSION

According to Schild's equation (Eq. 1) the shape of the curve of competitive antagonism should not be influenced by functional antagonism. The theory proposed in the preceding paper (3) suggests that the shape of the log $(X-1)$ vs. $-\log [B]$ curve of competitive antagonism varies markedly depending on the $U$ value. The term $U$ is multiplied $\phi$-fold by functional antagonism (Eq. 10), and the $\phi$ value depends on the concentration of a spasmogen used (Eq. 5). Then, the shape of the curve of competitive antagonism is predicted to vary depending on the concentration of a spasmogen used (functional antagonist). This was confirmed by the results shown in Figs. 1 and 2, where the shape of the curve of competitive antagonism between ISO and Prop in guinea-pig trachea is shown to be influenced by functional antagonism between ISO and a spasmogen (Cch or HA). According to the theory presented in this paper, the curve of competitive antagonism is influenced by functional antagonism when an agonist is removed from the region near the receptor by the saturable uptake process.
Care should be taken in evaluating the apparent pA₂ value by Schild's method when a spasmogen is used. Although Prop has generally been used as the reference compound in the research of β-adrenergic blocking agents, its apparent pA₂ values hitherto reported for guinea-pig tracheal preparation varied considerably as shown in the following: 6.56 (6), 7.90 (7), 8.02 (8), 8.17 (9), 8.31 (10), 8.45 (11), 9.15 (12), 9.20 (1), 9.76 (13). The smallest value shown above, 6.56, might be due to the use of noradrenaline as an agonist (noradrenaline is taken up more easily than ISO (14–16)). In the other cases (7–13), ISO was used. The variance of pA₂ of Prop from 7.90 to 9.76 may be for the greater part due to the application of Schild's equation for plotted points obtained under a variety of conditions. When Eq. 2 or Eq. 10 is applied, the pA₂ values of (±)-Prop obtained in this paper (Figs. 1 and 2) and previous paper (3) were always 9.20 regardless of the different experimental conditions (the presence or the absence of a spasmogen (HA or Cch), different concentrations of a spasmogens, or use of an uptake inhibitor (dibenamine)).

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