STUDIES ON MODE OF ANTAGONISM BETWEEN ADRENERGIC $\alpha$-MIMETICS AND $\beta$-BLOCKING AGENTS (I).  
$\beta$-BLOCKING ACTION OF MESCALINE AND ITS DERIVATIVES

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Abstract—In order to clarify whether or not trimetoquinol (TMQ) and isoproterenol (ISO) interact with the same receptor, the pA₂ values of propranolol (PR) and certain trimethoxybenzene derivatives were measured, using isolated guinea pig tracheal chains. Each of PR, mescaline (MES) and its derivatives gave almost the same pA₂ values for TMQ and ISO. Introduction of an alkyl group into the N atom of MES increased the affinity to the receptor in the order of methyl and isopropyl as well as the structure-activity relationship of catecholamines, while that of hydroxyl group in the 3-position of the side chain decreased pA₂ values. The slopes of the regression lines for anti-TMQ action of MES derivatives as well as PR were almost one, but those for their anti-ISO action were less than 0.3. 3,4,5-Trimethoxyaniline and 3,4,5-trimethoxybenzoic acid had little activity as $\beta$-blocking agents. These results suggest the possibility that TMQ and ISO would interact with the same receptor sites. The importance of the trimethoxybenzene and the phenethylamine moieties in the MES-derivatives for anti-TMQ action is discussed.

Adrenergic $\beta$-mimetics had been found almost exclusively in 3,4-dihydroxyphenylethanolamine derivatives, catecholamines (CA), until trimetoquinol and its derivatives were reported to be potent $\beta$-adrenergic stimulants (1). Trimetoquinol (TMQ) and its congeners have unique chemical structures compared with other $\beta$-mimetics such as isoproterenol (ISO).

In order to manifest the adrenergic $\beta$-action, TMQ and its congeners should possess the following chemical structures; i) 1,2,3,4-tetrahydroisoquinoline nucleus, ii) two hydroxyl groups at position 6 and 7 of the isoquinoline and iii) arylmethyl group at position 1 (1).

Although TMQ and ISO have the same moieties as shown with thick lines in Fig. 1, their chemical structures are quite distinctive from the viewpoint of structure-activity relationships. TMQ does not possess the $\beta$-hydroxyl group which is essential to manifest activities of ISO and its congeners (2, 3). Instead, TMQ

Fig. 1. Chemical structures of trimetoquinol (TMQ) and isoproterenol (ISO). The same moieties are shown with thick lines.
possesses the 3,4,5-trimethoxybenzyl group which would play an important role in its action (1). These dissimilarities in their chemical structures prompted us to compare their structure-activity relationships.

The structure-activity relationship should be discussed on the assumption that the compounds tested might interact with the same receptor site in the preparation used. This assumption could be easily accepted, if the compounds have almost similar chemical structures, in other words, belong to a certain series of compounds, like norepinephrine, epinephrine and isoproterenol. However, it should be determined whether or not TMQ and ISO interact with the same receptor sites before comparing their structure-activity relationships, since as described above, there were apparent structural differences between them.

As first pointed out by Schild (4, 5), pA values for a given antagonist acting competitively on a specific receptor in a given preparation should be the same regardless of the agonist used, if the agonists act on the same receptor. Thus, the determination of pA values provides a powerful method not only for classifying agonists according to the receptors on which they act, but also for classifying receptors on which a given agonist acts.

Accordingly, in this paper, pA2 values of some compounds, including adrenergic β-blocking agents, against bronchodilating actions of TMQ and ISO were measured in order to ascertain whether TMQ and ISO would act on the same receptor site. After confirming this point, structure-activity relationships of TMQ-series and catecholamines are discussed from the viewpoint of antagonism.

MATERIALS AND METHODS

Male guinea pigs of 250 to 400 g body weight were sacrificed by a blow on the head and bled from the femoral artery. The whole length of the trachea was removed, placed in Tyrode’s solution at room temperature and carefully cleaned of connective tissue. The trachea was then sectioned into 4 rings of equal width and each ring was opened by cutting through the cartilage opposite to the muscular portion. Along the cartilage each piece was trisected by two alternate cuts as long as approximately three quarters of its width. A tracheal chain was made by tying two tracheal pieces obtained from different parts of the trachea of different animals.

Two chains obtained from 4 guinea pigs were suspended in an organ bath filled with 20 ml of Tyrode’s solution at 36–0.5 °C into which air was continuously bubbled. The preparation was allowed to equilibrate for a period of approximately three hours. Cumulative dose-response curves to TMQ or ISO were determined in tracheal chains which were maximally contracted with histamine 2HCl (HA) at a final concentration of 1 × 10⁻⁵ g/ml. The preparation was washed with fresh Tyrode’s solution at an interval of about 10 min for 1 hr. A test compound was added to the bath 30 min prior to giving HA. Cumulative addition of the β-mimetics was begun approximately 15 min after addition of HA to the bath. The responses of the tracheal chain were recorded on a kymograph by means of an isotonic lever.

The stoichiometry of the drug-receptor interactions, in general, can be expressed as
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follows:

\[ m[A] + [R] = [A_mR] \]  
\[ n[B] + [R] = [B_nR] \]

where \([A]\) and \([B]\) are concentrations of the agonist and antagonist, respectively. \([R]\) is concentration of free receptors and \([A_mR]\) and \([B_nR]\) are concentrations of receptor-agonist and receptor-antagonist complex. Using the above formulae, the following equation can be reduced:

\[ \frac{[A]^m}{[A]^n} - 1 = \frac{[B]^n}{K_n} \]

Equation (3) when put in logarithmic form becomes:

\[ \log(X^m - 1) = n \log[B] - \log K_n \]  

where \(X (= [A]/[A])\) is the ratio of concentrations of agonist giving equal responses in the presence and in the absence of the antagonist, and \(K_n\) is dissociation constant of the receptor-antagonist complex. Based on analysis of the dose-response curves of the agonists, it can be concluded that \(m\) was 1 for both TMQ and ISO. Therefore, the following equation (5) was used to draw a regression line, using the method of least square.

\[ \log(X - 1) = n \log[B] - \log K_n \]

Values of logarithm of \((X - 1)\) were obtained by the method of Van Rossum (6) and plotted against negative logarithm of the concentration of antagonist tested, \(-\log[B]\). Since, for accurate determinations of \(pA_2\), it is desirable to obtain dose-response curves for the agonist over a wide range of antagonist concentrations, ranges not less than 100-fold of antagonist concentrations were employed. At least three experiments were carried out with an antagonist, and three doses of an antagonist were adopted in each experiment to construct the \(\log(X - 1)\) vs. \(-\log[B]\) curve. If the height of HA-induced contraction was reduced significantly by pretreatment with a test compound of a certain concentration, the experiment was no longer carried out at this concentration.

When the slopes of the regression lines were reasonably close to the theoretical value of 1, \(pA_2\) values were calculated by the method of Van Rossum (6) and expressed as mean of more than 12 experiments \(\pm S.E.M\). However, if considerably less than unity, they were obtained as intersections of lines with the abscissa.

The drugs used in the present work were as follows; trimetoquinol HCl (TMQ; Tanabe Seiyaku), (+)-isoproterenol HCl (ISO; Boehringer Sohn), propranolol HCl (PR; I. C. I.), and histamine 2HCl (HA; Tokyo Kasei).

All the other compounds were synthesized by the authors.

RESULTS

Effects of propranolol on trimetoquinol and isoproterenol

Effects of propranolol (PR) on bronchodilating activities of TMQ and ISO were investigated.
Fig. 2 shows the effects of different concentrations of PR on the dose-response curves of TMQ and ISO. Magnitude of the parallel shift of the dose-response curve of TMQ, caused by a certain concentration of PR, appeared to be almost equal to that of ISO. Strictly speaking, however, ISO was likely to be antagonized less than TMQ by PR. Plots of log (X−1) against negative log (concentration of PR) taken from the data in Fig. 2 were shown in Fig. 3. The slopes of the regression lines were 1.13 for TMQ and 1.10 for ISO, and they were not significantly different from the theoretical value, 1. Therefore, the pA₂ values of PR for TMQ and ISO were calculated with van Rossum’s method (6), and 9.07±0.11 and 8.96±0.07, respectively.

These results that the pA₂ values of PR for TMQ and ISO were almost the same suggests the possibility that the agonists used act on the same receptor site in the tracheal muscle preparation.

Effects of mescaline (MES) on TMQ and ISO

It can be said that TMQ is distinguished from ISO by having the trimethoxybenzyl group which would play an important role in formation of the drug-receptor complex. Since MES possesses this characteristic moiety, the antagonistic effects of MES on the bronchodilating actions of TMQ and ISO were investigated and shown in Fig. 4. Regression lines for TMQ and ISO intersected the abscissa at almost the same point. This means that
the pA₂ values of MES are almost the same regardless of the agonist used.

The higher the concentration of MES, the greater were the differences between the magnitudes of parallel shift of the dose-response curves for TMQ and ISO. Therefore, the regression line of MES with TMQ became steeper than that with ISO. The slope of the regression line for TMQ was 0.77, not significantly different from the theoretical value of 1, while that for ISO was 0.40.

**Effects of 3,4,5-trimethoxybenzene derivatives on TMQ and ISO**

In order to investigate the role of trimethoxybenzene moiety in TMQ on interaction with the receptor, certain compounds with 3,4,5-trimethoxybenzene moiety were examined as possible antagonists against TMQ and ISO, which were 3,4,5-trimethoxyaniline, 3,4,5-trimethoxybenzoic acid and 3,4,5-trimethoxybenzylamine.

The slopes of the regression lines of these three trimethoxybenzene derivatives for both TMQ and ISO were lower than 0.3. Dose-response curves for TMQ were shifted, if at all, wider than those for ISO. As shown in Fig. 5, even at such a high concentration as 10⁻³ M, 3,4,5-trimethoxyaniline and 3,4,5-trimethoxybenzoic acid shifted the dose-response curves for TMQ to the right by no more than 1 log unit, therefore, it could be concluded that they had little activity as β-blocking agents.

The regression lines of 3,4,5-trimethoxybenzylamine for TMQ and ISO were parallel as shown in Fig. 5. The remarkably large pA₂ value for TMQ, 9.66, was obtained as intersection of the line with the abscissa, while that for ISO was 6.41.
Effects of chemical modification of MES on the antagonistic actions

Referring to the well-established structure-activity relationships of catecholamines (3), methyl and isopropyl groups were introduced to the nitrogen atom of MES.

As to the anti-TMQ activity, introduction of the alkyl groups to MES increased the affinity to the receptor in the order of methyl and isopropyl (Fig. 6), as well as the structure \( \beta \)-mimetic activity relationships of catecholamines (3, 7, 8). The pA\(_2\) values of N-methyl- and N-isopropyl-MES were 7.24 and 7.81, respectively.

Introduction of an alcoholic hydroxyl group to the \( \beta \)-position in the side chain of N-isopropyl-MES, the most potent anti-TMQ compound in the MES derivatives tested, resulted in a marked decrease in the pA\(_2\) value (Fig. 6). The pA\(_2\) value for the anti-TMQ activity was 5.52.

As shown in Fig. 6, the slopes of their regression lines were not affected significantly by introduction of alkyl or \( \beta \)-hydroxyl groups to MES.

The pA\(_2\) values of each MES-derivative for anti-ISO action were almost equal to those for the anti-TMQ action. (Figs. 6 and 7). The slope of the regression line for the anti-ISO action of MES was not affected by these chemical modifications (Fig. 7).
DISCUSSION

From a chemical point of view, TMQ and its congeners are quite distinctive from catecholamines. It is of great interest that they were found to be effective as β-sympathomimetics, despite the absence of the hydroxyl group at position 4 in the isoquinoline nucleus that would correspond to the β-hydroxyl group in the side chain of catecholamines, although the β-hydroxyl group had been assumed to play an important role for the formation of the drug-receptor complex (2, 3). In contrast, trimethoxybenzene moiety in TMQ which was assumed to be important (1) in forming the drug-receptor complex is absent in catecholamines. However, TMQ and its congeners have a catechol moiety, a secondary nitrogen atom substituted with a "bulky" group, and a phenethylamine moiety in common with the β-mimetic catecholamines as shown in Fig. 1.

If more than two series of compounds do not interact with the same receptor site, a comparison of their structure-activity relationships is fruitless. Therefore, it should be clarified whether or not TMQ and ISO interact with the same receptor sites.

The pA2 values of certain compounds, including propranolol (PR) and trimethoxybenzene derivatives, were therefore compared for TMQ and ISO. Such was based on Schild's hypothesis (4, 5) that pA values for a given competitive antagonist should be the same regardless of the agonists used, provided these agonists interact with the same receptor.

The pA2 values for PR were practically the same for TMQ and ISO. Mescaline (MES) and its derivatives, N-methyl-, N-isopropyl- and N-isopropyl-β-hydroxy-MES, also gave almost the same pA2 values for TMQ and ISO.

Antagonistic effects of MES and its derivatives and their mode of action on the tracheal chain preparation were investigated, since MES contracted the preparation at the concentration range used. The contractile response was not abolished either by atropine or chlorpheniramine but was abolished by phentolamine. In agreement with Clement De Paul Lynch (9), it can be concluded that MES contracted the tracheal muscle preparation by interacting with α-adrenoceptors. Provided that MES antagonized functionally (10, 11) the relaxing effect of TMQ by means of its α-mimetic action, it should be equally effective against the relaxing effect of any β-mimetics. As shown in Figs. 4, 5, 6 and 7, however, ISO was antagonized less than TMQ by equivalent concentrations of each of MES and its derivatives. Furthermore, two of MES derivatives tested, N-isopropyl- and N-isopropyl-β-hydroxy-MES showed qualitatively the same antagonism as MES, while they did not contract the preparation at all. These results would indicate the possibility that MES and its derivatives were not functional but rather competitive antagonists against β-mimetics on the tracheal muscle preparations.

Based on the results described above, it can be concluded that TMQ and ISO would interact with the same receptor, irrespective of their chemical structural differences, therefore, it would be possible to compare the structure-activity relationship of catecholamines with that of TMQ and its congeners.

Based on the structure-activity relationship of TMQ and its congeners, the importance of the trimethoxybenzene during formation of the drug-receptor complex was outlined in
a previous paper (1). The importance of the trimethoxybenzene for the formation of the complex was recognized using some trimethoxybenzene derivatives, and this was also from a viewpoint of the antagonism.

MES did reveal antagonistic effects on the bronchodilating actions of both TMQ and ISO, although it was approximately one thousandth as active as PR, while 3,4,5-trimethoxyaniline and 3,4,5-trimethoxybenzoic acid were practically inactive. These results suggest that in order to manifest β-blocking action the trimethoxyphenethylamine moiety appears to be important. When discussing the mode of competitive antagonism between two compounds such as TMQ and MES at the receptor site, it must be taken into account that the interaction between the receptor and the compounds occurs three-dimensionally. Since a structure corresponding to MES is found in TMQ, it would be reasonable to consider that MES competes for the β-adrenoceptor with the corresponding structure in the TMQ-molecule indicated with thick lines in Fig. 8.

Interestingly 3,4,5-trimethoxybenzylamine gave characteristic regression lines; those for TMQ and ISO were parallel and they intersected the abscissa at fairly different points. Whether or not the nitrogen atoms of MES and of ISO interact with the same active site in the β-adrenoceptor was next considered.

As well as the structure-activity relationship of catecholamines, introduction of methyl and isopropyl groups into the nitrogen atom of MES increased the affinity to the β-adrenoceptor in the order of methyl and isopropyl. This would suggest the possibility that the nitrogen atom of MES would interact with the same active site in the β-adrenoceptor as that of ISO and also probably that of TMQ. It could be concluded that both the N atom and the trimethoxybenzene in the MES-derivatives were involved in the manifestation of anti-TMQ activity.

In catecholamines, the fact is widely accepted that introduction of an alcoholic hydroxyl group into the β-position of the side chain increases the affinity to the β-adrenoceptor (2, 3). If MES-derivatives would compete for the receptor with the part of TMQ molecule shown with thick lines in Fig. 1, the introduction of the hydroxyl group would increase the affinity as was the case of the catecholamines. However, this chemical modification decreased the $pA_2$ values of MES-derivatives remarkably. This divergence of the structure-activity relationships suggests that MES-derivatives would not compete for the β-adrenoceptor with the part of TMQ-molecule shown with thick lines in Fig. 1, but would compete for the receptor with the other part shown with thick lines in Fig. 8.

The regression lines of PR for both TMQ and ISO have slopes of almost unity, although TMQ was antagonized more than ISO by PR. It was noted that the slopes of the regression lines of MES-derivatives with TMQ as agonist were essentially unity, as expected from the receptor theory, but the slopes with ISO were definitely less than unity, reflecting the smaller
displacement of the dose-response curves of ISO. Furchgott (12) pointed out if a plot of \( \log (X-1) \) against \( \log [B] \) did not give a straight line with a slope of 1, then either the theoretical assumption on which the equations were based was wrong, or experimental conditions were not satisfactory for application of the equations. He also proposed the uptake saturation model to explain the divergence of experimental results from the predictions of receptor theory. As to the slopes of the regression lines shown in the figures, our data obtained in this work should be considered under the light of newly developed receptor kinetics.

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