ANTISYMPATHOMIMETIC EFFECTS OF Kö 1313 AND Kö 1366 IN GUINEA-PIG ATRIA

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Abstract—The antagonistic effects of two new β-receptors blocking agents, Kö 1313 and Kö 1366 against the chronotropic and inotropic responses to noradrenaline and tyramine were studied on the isolated right and left atria of guinea-pigs. From the $K_B$ values estimated by means of three different methods, it was found that the potencies of Kö 1366 were 5 to 10 times and 5 to 7 times those of Kö 1313 in the antagonism against chronotropic and inotropic effects of noradrenaline respectively. From the depression of maximum chronotropic and inotropic effects of tyramine in the presence of these agents, Kö 1366 was demonstrated to be approx. three times more potent than Kö 1313 in the antagonism against both effects of tyramine.

Kö 1313 ([dl-α-(2-hydroxy-3-(isopropylamino)-propoxy) benzonitrile hydrochloride) and Kö 1366 ([dl-α-(2-hydroxy-2-(tert-butylamino)-propoxy) benzonitrile hydrochloride) have been found to be effective and potent β-receptor blocking agents (1-3) as well as anti-arrhythmic agents (4) with a slightly intrinsic β-receptor stimulant component in their profile of the activity (2, 3). It was concluded that the β-receptor blocking activity of Kö 1366 was more potent than that of Kö 1313 in antagonism of the cardiac action of isoproterenol and sympathetic nerve stimulation (1-3).

In the present study the antagonism of Kö 1313 and Kö 1366 against the sympathomimetic action of noradrenaline and tyramine was investigated in the isolated guinea-pig atrium.

It is to be expected that the study of the interaction of the β-receptor blocking agents with these sympathomimetic amines may provide information, not only on the β-receptor blocking activity but also on other properties of the agents which may directly and indirectly influence the manifestation of the former activity.

METHODS

Guinea-pigs weighing 230 to 350 g were sacrificed by a blow on the head. The right or left atrium was isolated and suspended at 30°C in a 20 ml organ bath containing Tyrode solution (NaCl, 137; KCl, 4; MgCl$_2$, 1.2; CaCl$_2$, 2.4; NaHCO$_3$, 12; NaH$_2$PO$_4$, 0.4 and glucose, 5.5 in mM). The solution was aerated with a mixture of 95% O$_2$ and 5% CO$_2$. 

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The spontaneous beating right atrium was used to determine the chronotropic effects, whereas the electrically driven left atrium was used to determine the inotropic effects. A bipunctate platinum electrode delivered a threshold stimulus of 2 msec (3 Hz, ca. 1 V). The resting tension was adjusted to approx. 0.5 g. Heart rate and contractile force stabilized at 139.0±2.7 beats/min and 0.57±0.02 g (mean and standard error, n=30) during the period of 30 to 60 min. The cumulative dose-response curves for the chronotropic and inotropic effects of noradrenaline bitartrate (0.005, 0.02, 0.1, 0.5, 2, 10, 50 and 200 µg/ml) or tyramine monohydrochloride (1, 3, 10, 30 and 60 µg/ml) (refer to the salts) were determined in the right and left atria. After thorough washing, most of the preparations were immediately treated with either of the β-receptor blocking agents, Kö 1313 (10⁻⁸, 3×10⁻⁸, 10⁻⁷, 3×10⁻⁷, 7×10⁻⁷ or 10⁻⁶ g/ml) or Kö 1366 (10⁻⁸, 3×10⁻⁸, 10⁻⁷ and 3×10⁻⁷ g/ml), while the others were not. The dose-response curves were measured again as a second trial 30 to 40 min after washing. Significant inotropic and chronotropic effects of the β-receptor blocking agents alone were unobserved at this time. The dose-response curves for noradrenaline in the second trial were very similar to those in the first trial in the preparations which were not treated with the β-blockers, whereas the curves for tyramine were more or less steepled in the second trial as compared to the curves in the first trial. After correcting these changes in sensitivity, the dose-response curves in the presence of the β-blockers were plotted relative to the maximum response in the absence of the agents. The measurement of the dose-response curve was repeated for the third time in a few preparations, in order to observe the recovery process.
RESULTS

1) Antagonism between noradrenaline and Kö 1313 or Kö 1366

The dose-response curves of noradrenaline before and after Kö 1313 and Kö 1366 are shown in Fig. 1 A, B, C and D. Curves either for the inotropic or chronotropic effect
are shifted to the right with a slight tendency to steepen in accordance with the increasing concentration of the β-receptor blocking agents.

In order to test the competitive antagonism, \( \log(x-1) \) against \( -\log(B) \) was plotted according to the following formula (5):

\[
(A) \quad \log(x-1) = -\log(B) + \text{constant}
\]

**Fig. 2.** Plot of \( \log(x-1) \) vs. \( -\log(B) \). \( x=\text{ED}_{50} \) in the presence/\( \text{ED}_{50} \) in the absence of KÖ 1313 or KÖ 1366 and \( B = \text{molar concentration of the respective agent, calculated from the values in Fig. 1} \). Dotted lines are regression lines by conventional least squares methods and solid lines are those by least squares methods with unit slope constrained. Lines in the right side represent those for KÖ 1313 and lines in the left side, those for KÖ 1366.

A) the chronotropic effects in the right atrium,

B) the inotropic effects in the left atrium.
\[ \log(x-1) = \log(K_B + n \log(B)) \]
where \( x \) = dose ratio of the agonist, noradrenaline, to yield the half maximum response in the presence of the molar concentrations (B) of the antagonist, and \( n \) is a number of molecules of antagonist combining with the receptor site.

The least square estimates of \( n \) from the curves in Fig. 1 A-D were not very far from unity in all four cases (Table 1). \( K_B \) values were estimated from the horizontal intersection points of the regression lines in Fig. 2 A and B and are shown as \( 10^{-a} \) (\( a = -\log K_B \)) in Table 1. This Table also demonstrates two other ways of estimating \( K_B \), as presented by Waud and Parker (6), in order to correct the value for the finding that \( n \) is not exactly 1. These are:

\[ K_B = 10^{-a/n} \text{ or } K_B = 10^{\exp\left\{ \frac{\sum \log(B) - \sum \log(x-1)}{(N-1)} \right\}} \]

The latter formula was derived from the least square methods under the condition that \( n = 1 \). \( (N-1) \) is the number of points obtained. There were rather large differences between the \( K_B \) values estimated by these three methods as shown in Table 1. The relative values of \( K_B \) were, however, comparatively constant and \( K_B \)'s of Kö 1313 were 5 to 10 times those of Kö 1366 in the antagonism against chronotropic effects, and 5 to 7 times those in the antagonism against inotropic effects.

2) Antagonism between tyramine and Kö 1313 or Kö 1366

Dose-response curves for the chronotropic and inotropic effects of tyramine in the absence and presence of Kö 1313 or Kö 1366 are shown in Fig. 3 A and B. The maximum chronotropic and inotropic effects of tyramine were not attained in the presence of Kö 1313 or Kö 1366 and the dose-response curves became progressively flatter in correspondence with increasing concentrations of either blocker. Thus, the mechanism of antagonism seems to be a noncompetitive one, which was previously demonstrated by Benfey and Varma in the interaction of tyramine with propanolol or pronethanol (7, 8).

Table 2 shows the relative decrease in the maximum chronotropic and inotropic effects of tyramine in the presence of \( 10^{-7} \) and \( 10^{-8} \) g/ml of Kö 1313 and Kö 1366. The relative potency of Kö 1366 to Kö 1313 was estimated from the values in Table 2 according to the formula, \( M = \bar{x}_s - \bar{x}_u - \left\{ \bar{y}_s - \bar{y}_u \right\} / b_s \), where \( M \) is the log ratio of potency, \( \bar{x}_s, \bar{x}_u, \bar{y}_s, \bar{y}_u \) are the weighted means of the log dose and the mean responses to the agent \( s \) (Kö 1313) and the agent \( u \) (Kö 1366) and \( b_s \) the slope of the dose-response curve common with both agents (see Emmens (9)).

Thus, after conversion into molar basis, it was found that Kö 1366 was 2.9 times more potent than Kö 1313 in the antagonism against the chronotropic effects of tyramine in the right atrium and 3.5 times more potent in the antagonism against the inotropic effects in the left atrium.

The recovery of the antagonism against the effects of tyramine and noradrenaline was almost complete in the preparations treated with low concentrations of Kö 1313 and was very slight in those treated with high concentrations of Kö 1366 in the third trial in normal Tyrode solution.
FIG. 3. Antagonistic effects of Kö 1313 and Kö 1366 against chronotropic (A) and inotropic (B) effects of tyramine (μg/ml; abscissa) in the right (A) and left (B) atria of guinea-pigs. Each curve represents the mean values of three to six experiments. Control (tyramine alone), ●; Kö 1313, 10⁻⁸, ▲; 10⁻⁷ g/ml, ▼; and Kö 1366, 10⁻⁸, △; 10⁻⁷ g/ml, ▽. Mean responses in the presence of the β-receptor blocking agents, different from those of tyramine alone are denoted by † (P<0.01) and + (P<0.05).

<table>
<thead>
<tr>
<th>Agents (g/ml)</th>
<th>Percentage of the maximum chronotropic effects</th>
<th>Percentage of the maximum inotropic effects</th>
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<tbody>
<tr>
<td>Kö 1313</td>
<td></td>
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<tr>
<td>10⁻⁸</td>
<td>37.9±9.7</td>
<td>66.2±5.8</td>
</tr>
<tr>
<td>10⁻⁷</td>
<td>17.3±6.8</td>
<td>27.9±4.3</td>
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<tr>
<td>Kö 1366</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10⁻⁸</td>
<td>29.0±6.6</td>
<td>46.7±6.4</td>
</tr>
<tr>
<td>10⁻⁷</td>
<td>7.7±4.2</td>
<td>5.2±3.4</td>
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EFFECTS OF KO 1313 AND KO 1366

DISCUSSION

The present study compared the antagonistic effects of two new β-receptor blocking agents, Kö 1313 and Kö 1366, on the chronotropic and inotropic responses to noradrenaline and tyramine.

Both antagonistic effects of Kö 1313 and Kö 1366 against chronotropic and inotropic responses to noradrenaline are characterized by the steepening of the curves in accordance with the shift to the right, as shown in Fig. 1. This steepening of the curves was more evident in the inotropic response than in the chronotropic one and more so in the presence of high concentrations of Kö 1366 than in the presence of those of Kö 1313. Because the dose-response curves for isoproterenol shifted to the right in a nearly parallel way and the curves steepened after cocaine, Blinks (10) presumed that this steepening of the curves could be associated with the inhibition of noradrenaline uptake into sympathetic nerve endings. An alternative explanation is that the positive inotropic effects due to α-receptor stimulation by noradrenaline (11) may become more significant in the presence of the β-receptor blocking agents. Thus, it is interesting to determine whether or not the difference in the slope of the curves between Kö 1313 and Kö 1366 is correlated with the difference of potency in the inhibition of noradrenaline uptake or in the interaction with the α-receptor of the agents.

The potency ratios of Kö 1366 to Kö 1313, 5 to 10 for the chronotropic effects and 5 to 7 for the inotropic effects, obtained in the present experiments, are not much different from those obtained by Mylecharane and Raper (3) in the antagonism of the inotropic effects of isoproterenol in the left atrium of rabbits, by Engelhardt and Traunecker (1) in the antagonism of the tension development by isoproterenol by the intravenous route in anesthetized dog heart and by Baum et al. (2) in similar experiments in dogs using the parenteral route.

Benfey and Varma (8) reported the pA10 of propranolol for the inotropic effects of noradrenaline to be 6.4. The KB value, approx. $5 \times 10^{-4}$ M, calculated from this pA10, is of the same order as those of Kö 1313, shown in Table 1. A preliminary estimation by the author of the KB values of propranolol for the chronotropic effects of noradrenaline was also of the same order as those of Kö 1313 reported here (unpublished). Thus, the difference between the potencies of Kö 1313 and propranolol in respect to the antagonism against noradrenaline effects was not demonstrable here, though the greater potency of Kö 1313 over propranolol was reported by Baum et al. (2) in canine heart in situ in respect to the antagonism against isoproterenol effects. A feature which distinguishes Kö 1313 from propranolol is the lower potency of the direct negative inotropic effect of this agent (unpublished), whereas that which distinguishes Kö 1366 from propranolol is the greater potency of the β-receptor blocking activity of the agent. The difference between KB values in the chronotropic effects and those in the inotropic effects appears to be too slight to presume the existence of two separate receptor types mediating both of the responses, as was the case with propranolol by Blinks (10). The potency of Kö 1366 estimated from the changes in the maximum responses to tyramine was approx. 3 times
that of Kö 1313 in the antagonism against the chronotropic and inotropic effects of tyramine. The relative ratio of the $K_{B'}$ values of the dissociation constants of noncompetitive antagonist, which is to reduce the responses to half-maximum (12), roughly estimated from the values in Table 2, revealed also that Kö 1366 was approx. 3 times superior to Kö 1313 in antagonism against both effects of tyramine. Thus, it appears that the potency ratio of Kö 1366 to Kö 1313 in antagonism against tyramine effects is slightly less than that in the antagonism of noradrenaline effects in spite of the apparent difference in the mechanisms of antagonism.

Benfey and Varma (7, 8) suggested that the antagonism of the $\beta$-receptor blocking agents, pronethanol and propranolol against the inotropic effects of tyramine may derive from cross-tachyphylaxis due to the sympathomimetic property of the agents, although it may also be concerned with the specific $\beta$-receptor blocking property. Thus, the potency ratio of Kö 1313 to Kö 1366 against tyramine antagonism estimated here, can be presumed to relate more closely to the ratio of $\beta$-receptor stimulant potency, which is inherent to the agents (2, 3), than to the ratio of $\beta$-receptor blocking potency.

REFERENCES