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Rilmenidine Differs From Clonidine in that it Lacks Histamine-like Activity

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Abstract—Studies were carried out to determine whether rilmenidine, a recently introduced antihypertensive agent with a similar mechanism of action of clonidine, possesses histamine-like activity on tissues responding to histamine H_2-receptor agonists. In guinea-pig isolated atria, both histamine and clonidine caused concentration-dependent positive chronotropic effects which were blocked by the histamine H_2-receptor antagonist cimetidine (5 μM); in contrast, rilmenidine produced a concentration-dependent negative chronotropic effect which was not altered by cimetidine. In stilboestrol-treated rat isolated uterus contracted by KCl, histamine and clonidine produces concentration-dependent relaxations which were blocked by cimetidine (5 μM); rilmenidine also produced relaxation, but this was not affected by cimetidine (5 μM). These findings suggest that rilmenidine, unlike clonidine, does not activate histamine H_2-receptors in either guinea-pig atria or rat uterus.

Rilmenidine is a newly introduced antihypertensive agent which has a similar mechanism of action to clonidine in acting as an agonist on central α_2-adrenoceptors (Van Zwieten 1988), but it has the advantage that it lacks the main adverse effect of clonidine of causing sedation (Van Zwieten 1988; Koenig-Berard et al 1988).

Some α-adrenoceptor agonists of the clonidine type possess histamine-like activity in addition to acting on α-adrenoceptors (Csongrady & Kobinger 1974; McCulloch et al 1980; Kenakin & Angus 1981; Rubio et al 1982). Both rilmenidine and clonidine are suggested to be partial agonists at α_2-adrenoceptors (Medgett et al 1978; Medgett & Rand 1981; Li & Rand 1988), but the molecular structure of rilmenidine is different from that of clonidine. We set out to determine whether rilmenidine, like clonidine, possesses histamine-like activity. This was investigated in isolated preparations of guinea-pig atria and rat uterus which have proved to be of use in studies aimed at identifying and characterizing histamine H_2-receptors (Black et al 1972).

Materials and Methods

Guinea-pig atria

Guinea-pigs of either sex (300–500 g) were stunned by a blow to the head and exsanguinated. The hearts were rapidly removed and the atria were dissected free and mounted in an organ bath containing 5 mL of modified Krebs-Henseleit solution of the following composition (mmol L^{-1}): NaCl, 118; KCl, 4·7; $NaHCO_3$, 25; $MgSO_4$, 0·45; KH_2PO_4, 1·03; $CaCl_2$ 2·5; D-(+)-glucose, 11·1; disodium edetate, 0·067; ascorbic acid, 0·14. The solution in the organ bath and in the reservoirs supplying the organ bath was gassed with a mixture of 95% O_2 and CO_2 and maintained at a temperature of 37°C. The spontaneous atrial contractions were measured with an isometric strain gauge exerting a diastolic tension of 1 g and their force and rate were continually monitored on a Grass pen recorder. The isolated atria were allowed to equilibrate for 30 min before any experimental procedures were carried out. During this time the bathing solution was changed every 5 min.

After the period of equilibration, the atria were exposed to graded doses of agonists added cumulatively and changes in force and rate were recorded. Two consecutive concentration-response curves to each agonist were established in each preparation, the second being started 30 min after the first was completed. When the effect of an antagonist was studied, it was added 20 min before the second concentration-response curve was constructed and was present throughout the remainder of the experiments.

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Rat atria

Albino Wistar rats of either sex (200–300 g) were used. The protocol was the same as described for guinea-pig atria.

Rat uterine horn

Albino female Wistar rats (200–300 g) were treated with stilboestrol (1 mg kg^{-1} i.p.) 24 h before they were stunned by a blow to the head and exsanguinated. The uterine horns were quickly dissected free and suspended in an organ bath containing 8 mL of de Jalon solution of the following composition (m mol L^{-1}): NaCl, 154·0; KCl, 5·6; $CaCl_2$, 0·54; glucose, 2·68; $NaHCO_3$, 5·95. The solution was continuously bubbled with 95% O_2 and 5% CO_2 and maintained at 35°C. The uterine horn was attached to an isometric strain gauge exerting a resting tension of 1 g and tension was continually monitored on a Rikadenki pen recorder. The preparations were allowed to equilibrate for 30 min, during which the bathing solution was replaced every 5 min, before any experimental procedures were begun.

Activation of histamine H_2-receptors in the rat uterus results in relaxation. To observe this effect contractions were evoked by the addition of 56 mM KCl and graded doses of agonists were added cumulatively. The relaxant effect of each agonist was measured as the percentage inhibition of the KCl-induced contraction. Two consecutive concentration-response curves to each agonist were established in each preparation, the second being started 30 min after the first was completed. When the effect of an antagonist was studied,

it was added 20 min before the second concentration-response curve was established and was present throughout the remainder of experiments.

Materials

The following drugs were used: cimetidine (SK & F); clonidine hydrochloride (Boehringer Ingelheim); histamine acid phosphate (British Drug Houses); rilmenidine phosphate (Servier); stilboestrol (Jurox); tetraethylammonium chloride (TEA; Sigma).

Statistics

Data are given as means and standard errors. Means were compared by unpaired 2-tailed Student's *t*-tests. Regression lines were fitted to the linear portions of concentration-response curves from individual experiments by the method of least squares; the lines were tested for deviation from linearity and EC50 values were calculated when maximal effects were obtained. To assess the effect of an antagonist, the estimated dissociation constant (K_B) and hence the pA_2 of the receptor-antagonist complex was calculated by the method of Furchgott (1967). Probability values of less than 0·05 were considered to indicate statistical significance.

Results

Effects of histamine, clonidine and rilmenidine on guinea-pig isolated atria

The chronotropic effects of the three agonists used on isolated guinea-pig atria are shown in Fig. 1. Histamine (0·03–30 μM) and clonidine (1–300 μM) caused concentration-dependent increases in the rate of atrial contractions. The force of contraction was also increased, but this was not quantified in the present study. The EC50 values for histamine and clonidine were 0·72 ± 0·8 μM and 9·7 ± 0·9 μM, respectively. The maximal response to clonidine was about 25% of that to histamine. In contrast, rilmenidine (3–300 μM) caused concentration-dependent decreases in the atrial rate. The maximal response to rilmenidine on atrial rate was not reached even with the highest concentration used.

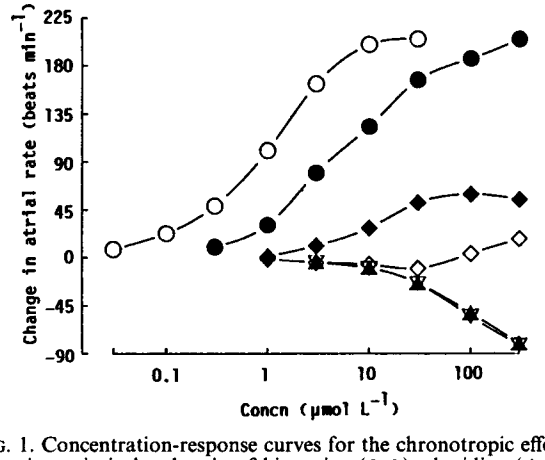

FIG. 1. Concentration-response curves for the chronotropic effects on guinea-pig isolated atria of histamine (○,●), clonidine (◆,◇) and rilmenidine (▽,▲) in the absence (○,◆,▽) and presence (●,◇,▲) of H_2-receptor antagonist cimetidine (5 μM). Symbols indicate mean values with n = 4–5 for each curve. Standard errors were smaller than the sizes of the symbols.

Effects of cimetidine on the chronotropic responses to histamine, clonidine and rilmenidine

The H_2-receptor antagonist cimetidine (5 μM) significantly shifted the concentration-response curve for histamine to the right without a change in the maximal response or the slope of the curve (Fig. 1). The estimated pA_2 value of cimetidine was 6·2. Cimetidine (5 μM) also significantly reduced the positive chronotropic response to clonidine, revealed a negative chronotropic effect (in the low concentration range), and suppressed the maximal response (in the high concentration range) (Fig. 1). The negative chronotropic effect of rilmenidine did not change in the presence of cimetidine (Fig. 1).

Effects of rilmenidine and clonidine on rat isolated atria

In rat atria, in contrast to guinea-pig atria, histamine did not have a chronotropic action. Both rilmenidine (3–300 μM) and clonidine (1–100 μM) caused concentration-dependent decreases in the atrial rate (Fig. 2). However maximal responses were not produced even by the highest concentrations used.

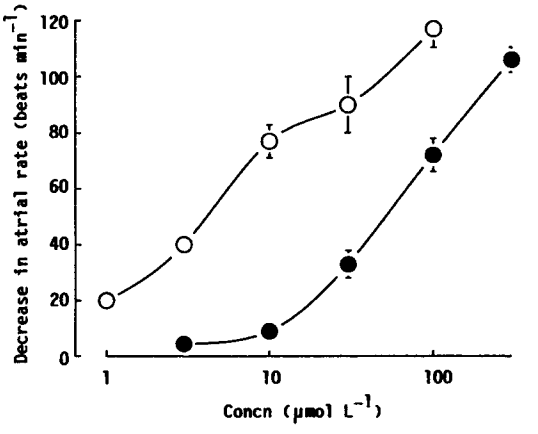

FIG. 2. Concentration-response curves for the chronotropic effects on rat isolated atria of clonidine (○) and rilmenidine (●). Symbols indicate mean values and standard errors with n = 5 for each curve.

Effects of other drugs on the negative chronotropic action of rilmenidine

The mechanism of the negative chronotropic effect of rilmenidine was further studied in both guinea-pig atria and rat atria using various other drugs. The effect of rilmenidine was not changed by atropine (3 μM), phentolamine (1 μM), 8-phenyltheophylline (3 μM), metoclopramide (1 μM) or indomethacin (5 μM) (results not shown), but were significantly reduced by TEA (3 mM) (Fig. 3).

Effects of rilmenidine, clonidine and histamine on rat isolated uterus

Clonidine (10–1000 μM) and rilmenidine (30–1000 μM), like histamine (3–1000 μM), caused relaxation of the stilboestrol-treated rat uterus, pre-contracted by 56 mM KCl. The concentration-response curves for these agonists are shown in Fig. 4. The EC50 value for the relaxant effect of histamine was 19·1 ± 0·4 μM, with the maximal effect at about 1000 μM; however, the EC50 values for clonidine and rilmenidine were

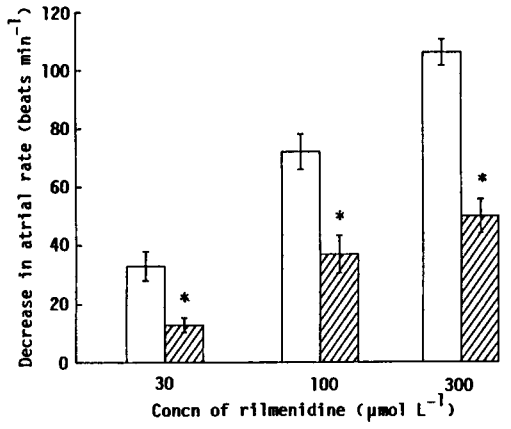

FIG. 3. Effect of TEA (3 mM) on the negative chronotropic response of rat isolated atria to rilmenidine. Control (□); TEA (■). Columns and bars represent means and standard errors with n = 4 for each. Asterisks (*) indicate significant differences ($P < 0.01$) between pairs of columns.

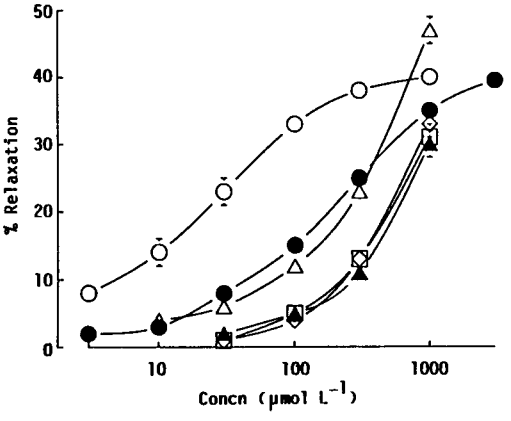

FIG. 4. Concentration-response curves for relaxant effects on rat isolated uterus contracted by 56 mM KCl of histamine (○,●), clonidine (△,◇) and rilmenidine (▲,□) in the absence (○,△,▲) and presence (●,◇,□) of the H_2-receptor antagonist cimetidine (5 μM). Symbols indicate mean values with n = 4 for each curve. Some standard errors were smaller than the sizes of the symbols.

not calculated because maximal responses were not obtained even with the highest concentrations used.

Effects of cimetidine on the relaxations of the rat uterus caused by histamine, clonidine and rilmenidine

Cimetidine (5 μM) significantly shifted the concentration-response curve for histamine to the right without a change in the maximal response or slope of the curve. The estimated pA_2 value of cimetidine was 6·1. The concentration-response curve for clonidine was also shifted to the right by cimetidine (5 μM), whereas that for rilmenidine did not change in the presence of cimetidine (Fig. 4).

Discussion

Histamine receptors are generally divided into three subtypes, namely H_1, H_2, and H_3 receptors (Schwartz et al 1986). The effects of histamine on gastric acid secretion, in relaxing the rat isolated uterus, and in increasing the rate and force of contractions of guinea-pig isolated atria are exerted on receptors of the H_2-subtype (Black et al 1972; Levi et al 1982). In the present study, the effects of histamine on guinea-pig atria and rat uterus were competitively antagonized by the H_2-receptor antagonist cimetidine, and the estimated pA_2 values (6·2 and 6·1) were consistent with those reported by previous workers (Bertacci & Coruzzi 1981; Brimblecombe et al 1975; Bradshaw et al 1979).

Some α-adrenoceptor agonists such as clonidine have been found to have histamine-like activity in both central and peripheral tissues, such as in guinea-pig brain slices (Audigier et al 1976), rat cerebral cortical neurones (Sastry & Phillis 1977), guinea-pig atria (McCulloch et al 1980; Kenakin & Angus 1981; Rubio et al 1982), rabbit aorta (Bokesoy et al 1978) and rat uterus (Parsons 1978). The histamine-like effects of clonidine on guinea-pig atria and rat uterus have been confirmed in the present study. In contrast to the effect of clonidine on guinea-pig isolated atria, rilmenidine had no histamine-like activity but had a negative chronotropic effect, which was not changed by cimetidine. The α-adrenoceptor agonists which possess histamine-like activity are imidazolidine derivatives. The studies of structure-activity relationship on these structural analogues of clonidine suggest that derivatives with 2,6-substitution on the phenyl ring are active as histamine H_2-receptor agonists, whereas derivatives with 2,3-, 2,4- or 2,5-substitutions are weakly active or inactive (McCulloch et al 1980). Rilmenidine is not an imidazolidine derivative therefore the lack of histamine-like activity suggests that the molecular structure of rilmenidine may not meet the functional requirement of agonists on histamine receptors (Ganellin 1982).

The mechanism of the negative chronotropic effect of rilmenidine is not clear. A similar effect was produced by clonidine after H_2-receptor blockade with cimetidine. Furthermore, both clonidine and rilmenidine had negative chronotropic actions in rat atria which is devoid of histamine receptors (Levi et al 1982). The findings suggest that the same mechanism may be involved in the negative chronotropic effects of rilmenidine and clonidine. As the negative chronotropic effect of rilmenidine was not affected by drugs that block α-adrenoceptors, muscarinic receptors, adenosine receptors, dopamine receptors and prostaglandin synthesis, the involvement of noradrenergic, cholinergic or dopaminergic mechanisms or release of endogenous adenosine or a prostaglandin could be excluded. Therefore, other possible mechanisms were considered, including local anaesthetic activity (Schmitt 1977), calcium channel blockade or potassium channel activation. Exploratory experiments with rat phrenic nerve hemidiaphragm and rat aorta preparations provided no evidence for apparent local anaesthetic activity or calcium channel blocking activity with the range of concentrations of rilmenidine used in the present study. However the potassium channel blocker TEA did decrease the negative chronotropic effect of rilmenidine on rat atria, suggesting that it may be due to activation of potassium channels, but further experiments are needed to elucidate the significance of this finding since the mechanism of the action of TEA is rather complex (Stanfield 1983). The concentration of rilmenidine required to elicit the negative chronotropic effect was relatively high, so it is unlikely that it would be exhibited with therapeutic doses.

Results obtained from rat isolated uterus experiments also

indicate that rilmenidine lacks histamine-like activity, since the relaxant effect of rilmenidine was not affected by cimetidine in the concentration which blocked the effects of histamine and clonidine. The histamine-like effect of clonidine has been confirmed in this study; however, the maximal effect of clonidine was not obtained, which differs from a previous report which stated that clonidine had approximately 2% of the potency of histamine as an agonist and gave about 50% of the maximal response to histamine (Parsons 1978). It is not clear whether a difference in methodology accounts for this discrepancy, as the methods used by Parsons (1978) were not described in detail. A complex action of clonidine on the rat uterus has been suggested (Parsons 1978). In addition to activation of H_2-receptors in the rat uterus, other mechanisms may be involved, especially with high concentrations. In our study, the concentration-response curve for clonidine in the presence of cimetidine and that for rilmenidine were nearly the same. This suggests that the effect is not due to actions of the drugs on α_2-adrenoceptors since clonidine is much more active than rilmenidine in this respect (Verbeuren et al 1986). The mechanism of the relaxation action of rilmenidine on the uterus was not further investigated.

The findings obtained in this study shows that rilmenidine, unlike clonidine, lacks histamine-like activity on either guinea-pig atria or rat uterus. This difference in the pharmacological spectrum of activity may be reflected in differences between the clinical effects of the two drugs. A dissociation between the sedative and antihypertensive effects of rilmenidine has been reported (Koenig-Berard et al 1988; Van Zwieten 1988). It has been suggested that clonidine-induced sedation is due to activation of central α_2-adrenoceptors (Timmermans et al 1981), as is the antihypertensive action. Since rilmenidine also acts on α_2-adrenoceptors to produce its antihypertensive action, it would be expected to produce sedation if the suggestion by Timmermans et al (1981) was correct. The possibility that the difference between clonidine and rilmenidine in histamine-like activity is implicated in the lack of a sedative effect with rilmenidine is worth considering. In support of this possibility, it has been shown that some imidazolidine derivatives, such as ST 600, which have weaker histamine-like activity than clonidine, have less sedative activity than clonidine (Hoefke et al 1975; Kho et al 1975; Malta et al 1980).

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