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STRUCTURE-ACTIVITY RELATIONSHIP OF IMIDAZOLIDINE DERIVATIVES RELATED TO CLONIDINE AT HISTAMINE H_2-RECEPTORS IN GUINEA-PIG ISOLATED ATRIA

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1 Cumulative concentration-response relationships for the chronotropic effects of histamine, oxymetazoline, clonidine and thirteen clonidine-like imidazolidine derivatives were examined in isolated spontaneously beating guinea-pig atria.

2 The following compounds induced positive chronotropic effects: histamine, clonidine (2,6-dichlorophenyliminoimidazolidine) and the 2,6-dibromo, 2,6-dimethyl, 2,6-diethyl, 2,6-dihydroxy, 2,4,6-trimethyl, 3,4-dihydroxy and 2-methyl-5-fluoro analogues of clonidine. These compounds appeared to act as partial agonists on histamine H_2-receptors, with potencies ranging from one tenth to one hundredth and intrinsic activities from approximately 20% to 75% of those of histamine.

3 The following compounds did not induce positive chronotropic effects but rather decreased the atrial rate, usually at high concentrations: oxymetazoline and the 2,3-dichloro, 4-dichloro, 5-dichloro, 2-chloro-4-methyl, 2-methyl-5-chloro, 2,4,6-trichloro analogues of clonidine.

4 The effects of histamine were antagonized by cimetidine, the pA_2 value being 6.68 (s.e. mean = 0.16, $n = 3$), and also in a concentration-dependent manner by clonidine. Cimetidine antagonized the response to clonidine in a concentration-dependent manner; however, high concentrations of cimetidine depressed the maximal response to clonidine and the slope of the concentration-response curve was no longer parallel to the control curve.

5 The effects of the other compounds which induced positive chronotropic effects were also antagonized by cimetidine (1 μmol/l); however, the effect of the 3,4-dihydroxy derivative was unaffected by cimetidine (1 μmol/l) but was abolished by propranolol (0.3 μmol/l).

6 In general, phenyliminoimidazolidine derivatives with 2,6-substitution on the phenyl ring are active on histamine H_2-receptors, whereas derivatives with 2,3-, 2,4- or 2,5-substitutions are weakly active or inactive. Thus the restriction imposed on the free rotation of the phenyl ring about the carbon-imino nitrogen bond by the introduction of two ortho substituents appears to result in increased agonist activity on the histamine H_2-receptor. The introduction of substituents in the 3, 4 or 5 positions in the phenyl ring may lead to compounds sterically hindered from combining with the histamine H_2-receptor.

7 There is no apparent relationship between the activities of clonidine-like imidazolidine derivatives as agonists on histamine H_2-receptors and their hypotensive activities (as reported in the literature).

Introduction

The mode of action of the potent centrally-acting antihypertensive drug clonidine has generally been considered to be due to activation of central α-adrenoceptors (for a review, see Van Zwieten, 1977). However, clonidine has been shown to activate histamine H_2-receptors in the isolated, perfused heart of the guinea-pig (Csongrady & Kobinger, 1974), and several workers have now reported that the central administration of histamine H_2-receptor blocking drugs interferes with the hypotensive effects of clonidine (Karppannen, Paakkari, Paakkari, Huotari & Orma, 1976; Paakkari, Paakkari & Karppannen, 1976; Finch, Harvey, Hicks & Owen, 1977). It has been suggested, therefore, that central histamine H_2-receptors are involved in the hypotensive response to clonidine. On the other hand, we have previously found (McCulloch, Medgett & Rand, 1979) that the H_2-receptor blocking drugs, burimamide and cimetidine, possess weak α-adrenoceptor blocking activity which may account for the central interaction between these blocking drugs and clonidine.

Further evidence concerning the involvement of

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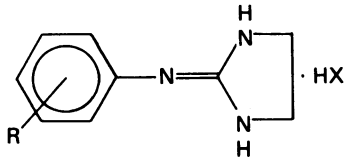

Figure 1 General structure of imidazolidine derivatives used in this study.

central histamine H_2-receptors in the hypotensive effect of clonidine may come from a comparison of structure-activity requirements for hypotensive activity with those for activation of histamine H_2-receptors. This aspect has not previously been considered in the several reports that have been published in recent years examining the hypotensive and bradycardic effects of structural analogues of clonidine in order to determine their structure-activity relationships (Struyker Boudier, Smeets, Brouwer & van Rossum, 1974; Hoefke, Kobinger & Walland, 1975; Jen, Van Hoeven, Groves, McLean & Loev, 1975; Timmermans & van Zwieten, 1977a, b, c). Consequently, the present study is concerned with the effects of clonidine and fourteen structural analogues on the rate of spontaneous beating of guinea-pig isolated atria; this tissue was used in the original studies aimed at identifying and characterizing histamine H_2-receptors (Black, Duncan, Durant, Ganellin & Parsons, 1972).

The general structure of the imidazolidine derivatives used is shown in Figure 1. All the compounds except oxymetazoline are phenyliminoimidazolidines, di- or tri-substituted in the phenyl ring. A preliminary account of this work was given to the Australian Physiological and Pharmacological Society (Medgett & McCulloch, 1978).

Methods

Guinea-pig atria

Guinea-pigs of either sex weighing 300 to 500 g were killed by cervical dislocation and exsanguinated. The hearts were rapidly removed and the atria were dissected free and suspended in a 2.5 ml jacketed organ bath containing Krebs-Henseleit solution of the following composition (mmol/l): 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 and sodium edetate 0.67. The solution in the bath was continuously gassed with a mixture containing 95% O_2 and 5% CO_2. The spontaneous beating of the atria was measured with a high compliance strain gauge transducer, exerting an initial tension of about 1 g. The output was used to trigger a cardiotachometer and the rate and force of atrial beating were simultaneously displayed by a Brush mark 250 pen recorder. After an equilibration period of 1 h, during which the bathing solution was repeatedly exchanged for fresh Krebs-Henseleit solution approximately every 5 min, a cumulative concentration-response curve to the chronotropic effects of histamine, oxymetazoline or one of the imidazolidine derivatives was obtained. Chronotropic effects are presented as changes from the resting rate of beating. Second and subsequent curves were obtained only after complete recovery from the effects of the preceding drug addition. At least three curves were obtained from each atrial preparation. In experiments where the effects of an antagonist drug were assessed, the concentration-response curve to the agonist drug was obtained first and subsequent curves were then obtained in the presence of successive concentration increments of the antagonist drug, which was present in the bathing solution at the concentration required for at least 15 min before re-exposure of the tissue to the agonist drug.

Drugs

The following drugs were used: atropine sulphate (David G. Bull); cimetidine base (Smith, Kline & French); histamine acid phosphate (B.D.H.); oxymetazoline hydrochloride (Glaxo); phentolamine mesylate (Ciba); propranolol hydrochloride (ICI). The following phenyliminoimidazolidine derivatives were used; the substituents on the phenyl ring (see Figure 1) are given in brackets: clonidine hydrochloride (2,6-dichloro); St 89 hydrochloride (2,4,6-trimethyl); St 91 hydrochloride (2,6-diethyl); St 95 hydrochloride (2,6-dimethyl); St 363 hydrochloride (2,4-dichloro); St 375 nitrate (2-chloro-4-methyl); St 464 (base; 2,6-dibromo); St 475 hydrochloride (2,5-dichloro); St 476 hydrochloride (2,3-dichloro); St 585 hydrochloride (2-methyl, 5-chloro); St 600 hydrochloride (2-methyl-5-fluoro); St 732 hydrochloride (2,4,6-trimethyl); St 1943 hydrobromide (3,4-dihydroxy); St 1946 hydrobromide (2,6-dihydroxy). The imidazolidine derivatives were supplied by Boehringer Ingelheim. All drugs were initially dissolved in distilled water except for the bases which were dissolved in the minimum volume of 0.1 M HCl; in the case of the 3,4-dihydroxy derivative, the distilled water contained sodium edetate (50 μg/ml) and ascorbic acid (50 μg/ml) to retard oxidation of the catechol group. Final dilutions were made in Krebs-Henseleit solution.

Analysis of results and statistics

Regression lines were fitted to the linear portions of concentration-response curves from individual experiments, by the method of least squares; the lines were

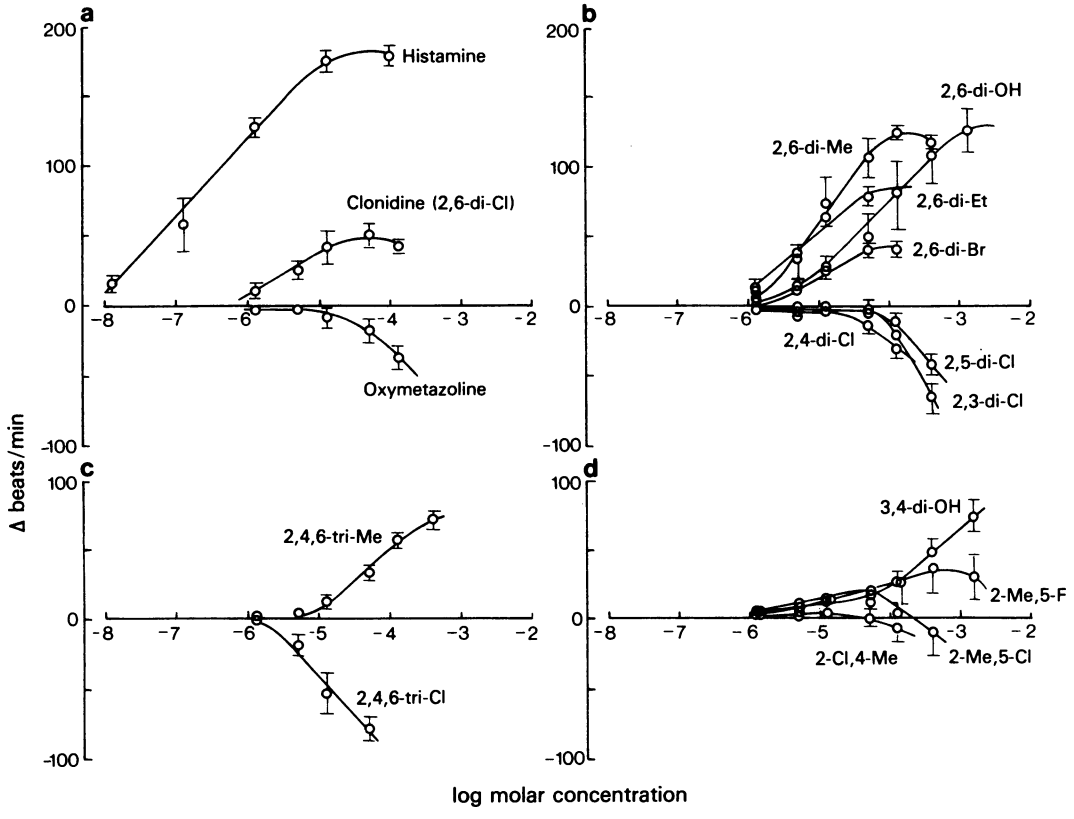

Figure 2 Cumulative concentration-response curves to the chronotropic effects of histamine, clonidine (2,6-dichloro derivative) and oxymetazoline (a); 2,3-, 2,4- and 2,5-dichloro, 2,6-dihydroxy, dimethyl, diethyl and dibromo derivatives (b); 2,4,6-trimethyl and trichloro derivatives (c) and the 3,4-dihydroxy, 2-methyl-5-chloro, 2-methyl-5-fluoro and 2-chloro-4-methyl derivatives (d). Each point represents the mean of at least three determinations; the vertical bars represent s.e. means. Ordinate scale: change in atrial rate, expressed as change in the number of beats per min. Abscissa scale: logarithm of the molar concentration of the drug. In contrast to the other compounds, the effects of oxymetazoline and 2,4,6-trichlorophenyliminoimidazolidine were not readily reversible on washout, thus the curves in the figure show the effects of the first exposure of the atria to the drug (see Methods).

tested for deviation from linearity and EC_{50} values (concentrations of agonists required to produce 50% of their own maximum effect) were calculated. To assess the effect of an antagonist, when only one antagonist concentration was used, the dissociation constant (K_B) of the receptor-antagonist complex was calculated by the method of Furchgott (1967); when several concentrations of antagonist were used, the type of antagonism and the pA_2 value were determined by the method of Arunlakshana & Schild (1959).

Results

Chronotropic effects of histamine, oxymetazoline and phenyliminoimidazolidine derivatives

Figure 2a shows the effects of histamine, clonidine (the 2,6-dichloro derivative) and oxymetazoline (2-(4-*tert*-butyl-2,6-dimethyl-3-hydroxybenzyl)-2-imidazoline) on the rate of beating of guinea-pig isolated atria. Histamine and clonidine increased the rate of beating, clonidine being less potent and having a

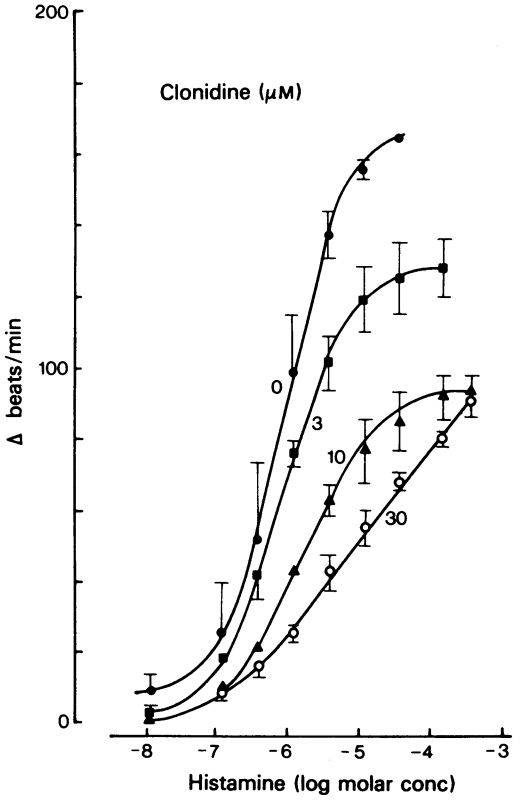

Figure 3 Cumulative concentration-response curves to histamine in the absence and presence of clonidine (3 μmol/l to 30 μmol/l). See legend to Figure 2 for further details.

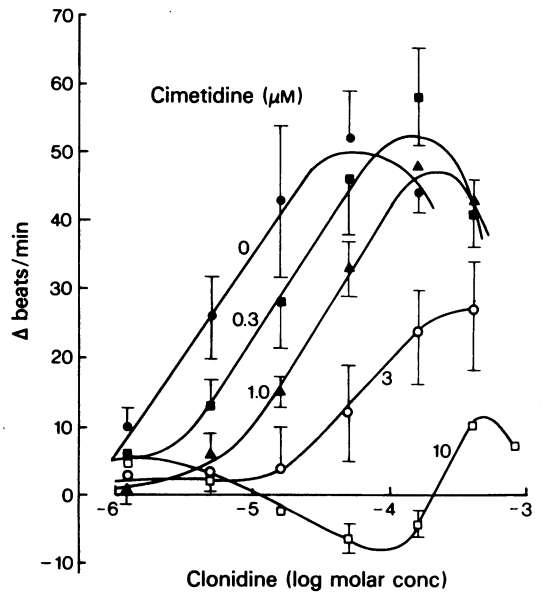

Figure 4 Cumulative concentration-response curves to clonidine in the absence and presence of cimetidine (0.3 μmol/l) to 10 μmol/l). See legend to Figure 2 for further details.

lower maximal effect than histamine. In contrast, oxymetazoline had negative chronotopic activity which was only seen in concentrations higher than 10 μmol/l. The positive chronotropic effect of clonidine was unchanged in the presence of a combination of phentolamine (0.1 μmol/l), propranolol (0.3 μmol/l) and atropine (0.1 μmol/l).

Figure 2b shows that the other derivatives tested which were 2,6-disubstituted in the phenyl ring (dibromo, dimethyl, diethyl and dihydroxy derivatives), like clonidine increased the rate of beating of atria, in each case the maximal effect being less than that of histamine. The structural isomers of clonidine, the 2,3-, 2,4- and 2,5-dichloro derivatives, like oxymetazoline produced only negative chronotropic effects in high concentrations.

The effects of the two trisubstituted derivatives (2,4,6-trimethyl and trichloro derivatives) used in the study are shown in Figure 2c. The trimethyl derivative induced positive chronotropic effects whereas the trichloro derivative had marked negative chronotropic activity and was the most potent compound used in the study in this respect. The negative chronotropic effect of the trichloro derivative was unaltered in the presence of phentolamine (0.1 μmol/l), propranolol (0.3 μmol/l) and atropine (0.1 μmol/l).

As shown in Figure 2d, the 2-chloro-4-methyl, 2-methyl-5-chloro and 2-methyl-5-fluoro derivatives were either very weakly active or inactive with respect to positive chronotropic activity. The 3,4-dihydroxy derivative induced positive chronotropic effects although the concentration-response curve had not reached a plateau at the highest concentration tested.

Antagonism of positive chronotropic effects

The concentration-response curve to histamine was displaced to the right in a parallel and concentration-dependent manner by the histamine H_2-receptor blocking drug, cimetidine. The plot of log (dose ratio − 1) versus log antagonist concentration yielded a straight line with a gradient not significantly different from unity, suggesting that the antagonism was competitive. The pA_2 value was 6.68 (s.e. mean = 0.16, n = 3).

The positive chronotropic effect of histamine was also antagonized by clonidine. As shown in Figure 3 the concentration-response curves to histamine were

displaced to the right in a concentration-dependent manner by clonidine. In the presence of clonidine the maximum response to histamine was reduced and the curves obtained were not parallel to the control curve. In these experiments clonidine was added to the fluid bathing the atria at least 15 min before the second and subsequent histamine concentration-response curves were established. Clonidine had an initial positive chronotropic effect but this was not maintained and when the first of the subsequent doses of histamine was tested the rate of atrial contractions had stabilized at the preclonidine level.

Figure 4 shows that the clonidine concentration-response curve was displaced to the right in a concentration-dependent manner by cimetidine. With each of the two lowest cimetidine concentrations (0.3 and 1 μmol/l) the shift was parallel and there was no depression of the maximum response, suggesting competitive antagonism. However, with higher cimetidine concentrations (3 and 10 μmol/l) the shift was no longer parallel and there is a marked reduction in the maximum response. In the presence of the highest cimetidine concentration used (10 μmol/l), a slight negative chronotropic effect of clonidine was revealed.

Cimetidine (1 μmol/l) also displaced to the right the concentration-response curves of the 2,6-dibromo, 2,6-dimethyl and 2,6-dihydroxy derivatives; there was no significant departure from parallelism with the control curves in any case. Table 1 gives the pD_2 (negative logarithm of the molar EC_{50}) values for histamine, clonidine and the 2,6-dibromo, 2,6-dihydroxy and 2,6-dimethyl derivatives in the presence and absence of cimetidine (1 μmol/l) together with the values of the shift to the right for the concentration-response curves in log units and the K_B values for the antagonism by cimetidine of each of the compounds. The log shift and K_B values were similar for the five compounds suggesting a common receptor site of action for each of the agonists and cimetidine. Cimetidine (1 μmol/l) also antagonized the effects of the 2,6-diethyl, 2,4,6-trimethyl and the 2-methyl-5-fluoro derivatives although the antagonism did not appear competitive, since the displaced agonist concentration-response curves were not parallel to the control curves and there were large reductions in the maximum responses.

In contrast to the findings with the other imidazolidine derivatives tested, the positive chronotropic effect of the catechol derivative (3,4-dihydroxy) was unaffected by cimetidine (1 μmol/l); however, the effect was completely abolished by the β-adrenoceptor blocking drug, propranolol (0.3 μmol/l).

Correlation of the chronotropic activity of the compounds in isolated atria with their in vivo *cardiovascular activity and with their physicochemical parameters*

Table 2 summarizes data obtained in the present study for the chronotropic activity of histamine, oxymetazoline and the imidazolidine derivatives in guinea-pig isolated atria; the pD_2 and intrinsic activity values are given for compounds which induce positive chronotropic effects. Included in Table 2 for comparison with the present data are data for the hypotensive and bradycardic effects of nine of the imidazolidine derivatives in the anaesthetized normotensive rat from the study of Timmermans & van Zwieten (1977a). Also included in Table 2 are log P (logarithm of the octanol/buffer partition coefficient at pH 7.4), pK_A and log MV (logarithm of the molar volume of the phenyl ring and its substituents in the imidazolidine molecule). These three parameters give first approximations respectively to the hydrophobic, electronic and steric characteristics of the compounds,

Table 1 Negative logarithms of molar EC_{50} (pD_2) values for histamine, clonidine, 2,6-dibromo-, dihydroxy- and dimethylphenyliminoimidazolidine on histamine H_2-receptors in the absence (control) and presence of cimetidine (1 μmol/l)

	pD_2				
Agonist	*Control*	*Cimetidine*	*log shift*	K_B	n
Histamine	6.39 ± 0.05	5.81 ± 0.02	0.58 ± 0.01	4.3×10^{-7}	3
2,6-di-Cl (Clonidine)	5.31 ± 0.11	4.86 ± 0.05	0.45 ± 0.04	2.1×10^{-7}	3
2,6-di-Br	4.93 ± 0.07	4.51 ± 0.25	0.42 ± 0.07	6.2×10^{-7}	3
2,6-di-OH	4.09 ± 0.05	3.68 ± 0.09	0.41 ± 0.03	8.2×10^{-7}	3
2,6-di-Me	4.69 ± 0.04	4.20 ± 0.04	0.49 ± 0.01	3.6×10^{-7}	3

For the antagonistic effect of cimetidine, log shift (shift to the right of the concentration-response curve in log units as measured by the ratios of the EC_{50} values in the presence and absence of cimetidine) and K_B values (dissociation constant for the receptor-antagonist complex, calculated according to Furchgott, 1967) are given. Values are given as the mean ± s.e. mean; *n* is the number of experiments.

which are considered by Hansch (1973) to be of prime importance in quantitative structure-activity studies.

Discussion

Eight of the imidazolidine derivatives investigated in the present study had positive chronotropic effects in guinea-pig isolated atria. Five other derivatives had either no significant effect on the rate of beating of atria or induced negative chronotropic effects. No attempt was made to quantify inotropic effects of the imidazolidine derivatives; however, in general, these paralleled the chronotropic effects of the compounds, although, as has been pointed out by Steinberg & Holland (1975), increases in rate in spontaneously beating atrial preparations may be expected to lead directly to increases in force of beating.

The compound 3,4-dihydroxyphenyliminoimidazolidine exerted weak positive chronotropic effects which were unchanged in the presence of cimetidine but were abolished by propranolol, indicating that this compound activates cardiac β-adrenoceptors rather than histamine H_2-receptors. This is not surprising in view of the presence of the catechol group in the molecule; in addition, this compound has been reported to be a potent agonist at dopamine receptors in the brain of the snail *Helix aspersa* (Struyker Boudier, Teppema, Cools & van Rossum, 1975). Furthermore, in our laboratory it has been shown that all the imidazolidine derivatives used in the present study and oxymetazoline activate both postjunctional (guinea-pig aorta) and prejunctional (guinea-pig atria) α-adrenoceptors (Medgett, McCulloch & Story, 1979; McCulloch & Medgett, 1979).

With the exception of the 3,4-dihydroxy derivative, all the imidazolidines which exerted positive chronotropic effects appeared to act as partial agonists at histamine H_2-receptors in the atria. Thus the compounds exerted positive chronotropic effects with maxima less than that of histamine and these effects, like that of histamine, were antagonized by the histamine H_2-receptor antagonist cimetidine. The pA_2 value of 6.68 (s.e. mean = 0.16, $n = 3$) for the antag-

Table 2 Summary of data for chronotropic effects of histamine, clonidine, clonidine analogues and oxymetazoline in guinea-pig isolated atria

	Chronotropic effect in guinea-pig isolated atria					*Hypotensive potency in anaesthetized rat†*	*Physicochemical properties*		
+	−	0	pD_2	IA	n	$-\log ED_{30}$ (¶g/kg)	log P	pKa	log MV
Histamine			6.21 ± 0.16	1.00	6	n.a.	–	–	–
Clonidine			5.3 ± 0.11	0.30 ± 0.04	3	1.99	0.48	8.20	1.85
	Oxymetazoline		n.c.	n.c.	3	n.a.	−0.46	–	2.09
2,6-di-Me			4.90 ± 0.14	0.69 ± 0.04	3	0.85	−1.40	10.50	1.86
2,6-di-OH			3.90 ± 0.20	0.74 ± 0.10	6	n.a.	−3.00	7.73	1.80
2,6-di-Et			5.20 ± 0.13	0.48 ± 0.10	4	n.a.	−1.20	10.61	1.98
2,6-di-Br			4.93 ± 0.07	0.24 ± 0.03	3	1.81	1.17	8.20	1.88
	2,4-di-Cl		n.c.	n.c.	3	0.64	0.45	8.65	1.85
	2,5-di-Cl		n.c.	n.c.	3	0.25	0.79	8.50	1.85
	2,3-di-Cl		n.c.	n.c.	3	1.31	0.53	8.55	1.85
2,4,6-tri-Me			4.36 ± 0.07	0.34 ± 0.03	3	−0.07	−2.30	10.78	1.94
	2,4,6-tri-Cl		n.c.	n.c.	3	1.16	1.44	6.96	1.91
3,4-di-OH*			n.c.	n.c.	4	n.a.	−1.60	–	1.80
2-Me,5-F			3.94 ± 0.23	0.19 ± 0.08	4	n.a.	−0.80	9.98	1.80
		2-Cl,4-Me	n.c.	0.05 ± 0.04	3	0.67	−0.96	9.41	1.85
		2,-Me,5-Cl	n.c.	0.15 ± 0.02	3	n.a.	−0.30	9.50	1.85

* See text.

† These data taken from Timmermans & van Zwieten (1977a).

+ indicates that a positive, − a negative and 0 no chronotropic effect was induced by the compound indicated either by name or by the substitution on the phenyl ring as in Figure 1. pD_2 and intrinsic activity (IA) values are given as the mean ± standard error of the mean; n is the number of experiments. 'n.c.' indicates that a pD_2 or IA value was not calculated. Values are quoted for the hypotensive activity of the compounds in the unanaesthetized, normotensive rat (Timmermans & van Zwieten, 1977a). 'n.a.' indicates that the effects of a particular compound were not assessed in the study. Values of log P and pK_A were supplied by Boehringer Ingelheim (Australia). Values of log MV are for the molar volume of the phenyl ring and its substituents; this first approximation to the steric effect of the molecule has also been used by Struyker Boudier, de Boer, Smeets, Lien & van Rossum (1975). The van der Waal's volumes of the phenyl ring and its substituents were taken from the data of Bondi (1964).

onism by cimetidine of the effect of histamine agrees fairly well with the value of 6.1 reported by Brimblecombe, Duncan, Durant, Ganellin, Parsons & Black (1975) for guinea-pig isolated right atrial preparations. In antagonizing the positive chronotropic effects of clonidine, cimetidine, in concentrations higher than 1 μM, depressed the maximum response to clonidine. Sanders, Miller & Patil (1975) found a similar reduction in the maximum positive chronotropic response of guinea-pig atria to the imidazolidine derivatives tolazoline and naphazoline in the presence of the histamine H_2-receptor blocking drug, metiamide, and interpreted these results to mean that the imidazolines were acting as partial agonists at histamine H_2-receptors. In the present study, cimetidine displaced to the right in a parallel manner the concentration-response curves to 2,6-dibromo-, 2,6-dihydroxy- and 2,6-dimethylphenyliminoimidazolidine. The similar log shift and K_B values shown in Table 1 for the antagonism by cimetidine of the positive chronotropic effects of histamine and the imidazolidine derivatives suggest that the agonists share a common receptor site of action (Furchgott, 1967).

Clonidine-like imidazolidine derivatives have been reported not to activate β-adrenoceptors and also to possess some local anaesthetic and anticholinoceptor activity (see reviews by Schmitt, 1977; Kobinger, 1978). In guinea-pig isolated heart, the positive inotropic effect of clonidine has been shown to be antagonized by burimamide, a histamine H_2-receptor blocking drug but unaffected by β-adrenoceptor, α-adrenoceptor and histamine H_1-receptor blockade by doberol, phentolamine and pheniramine, respectively (Csongrady & Kobinger, 1974). In the present study, the positive chronotropic effect of clonidine was unaltered when the experiments were carried out in the presence of β-adrenoceptor, α-adrenoceptor and muscarinic receptor blockade by a combination of the drugs propranolol, phentolamine and atropine. The negative chronotropic effect of the 2,4,6-trichloro-derivative was also unaffected by these drugs. These experiments thus suggest that the chronotropic effects of these imidazolidine derivatives are not mediated by α- or β-adrenoceptors, or muscarinic receptors.

Table 2 shows that the potencies of the imidazolidine derivatives which had positive chronotropic activity (except the 3,4-dihydroxy derivative) ranged from approximately one tenth to one hundredth and their intrinsic activities from 20% to 75% of those of histamine. On the other hand similar threshold concentrations for positive chronotropic activity, shown in Fig. 2, would suggest that these imidazolidine derivatives all have similar affinities for histamine H_2-receptors.

In the present study, clonidine antagonized the positive chronotropic effects of histamine and also depressed the maximum response to histamine. The displacement of the histamine concentration-response relationship by clonidine is consistent with a partial agonist action of clonidine at histamine H_2-receptors. An agonistic action of clonidine at histamine H_2-receptors would be expected to lead to a reduction in the maximum response to histamine. However, the positive chronotropic action of clonidine was not maintained such that histamine concentration-response curves obtained in the presence of clonidine were not superimposed on an elevated baseline. It is possible that in the continued presence of clonidine, the depression of the maximum response to histamine may be due to the negative chronotropic effect of clonidine which was revealed in the presence of cimetidine (see Figure 4).

The mechanism of the negative chronotropic effects produced by some of the imidazolidines is not clear. The local anaesthetic effect of clonidine is similar to that of procaine (Hoefke & Kobinger, 1966); thus it is possible that the imidazolidine derivatives are exerting non-specific depressant effects on the atrial smooth muscle. It is interesting that only those imidazolidine derivatives which are lipid soluble (i.e., with positive log P values, see Table 2) have negative chronotropic effects. In this connection it may be noted that the 2,4,6-trichloro derivative, which was the most potent in inducing negative chronotropic effects (Figure 2), possessed the highest log P value in the series; in addition, this compound possessed the lowest pK_A (6.96) which indicates that at physiological pH, a high proportion of the molecules ($\simeq 80\%$) will be in the non-ionized (lipid soluble) state. Two of the imidazolidines did not significantly affect the beating of guinea-pig atria (2-chloro-4-methyl and 2-methyl-5-chloro derivatives); their negative log P values and high pK_A values probably account for their lack of negative chronotropic activity. Clonidine has a positive log P value and it is of interest that a slight negative chronotropic effect of clonidine is manifested in the presence of the highest concentration of cimetidine used (10 μmol/l). The negative chronotropic effect of the imidazolidine derivative, oxymetazoline, may appear surprising considering its negative log P value. In the study of Sanders *et al.* (1975) this effect of oxymetazoline was also observed and James, Bear, Lang & Green (1968) have provided evidence for the presence of α-adrenoceptors mediating decreases in the rate in the sinus node of the dog; however, the observation that the negative chronotropic effect of the 2,4,6-trichloro derivative was unaltered in the presence of the α-adrenoceptor blocking drug, phentolamine, appears to discount the possibility that the negative chronotropic effects of imidazolidine derivatives are due to an α-adrenoceptor agonist action.

Since the different slopes of the concentration-response curves (Figure 2) clearly reflect for the lipid

soluble derivatives the sum of both positive and negative chronotropic activity, it is not possible to correlate precisely the physicochemical parameters of the imidazolidine derivatives with their ability to act as partial agonists on atrial histamine H_2-receptors. However, some general conclusions may yet be drawn from these data. Thus, the lipid solubility of the compounds does not appear to be positively correlated with their histamine H_2-receptor agonist activity in guinea-pig atria, nor is there any obvious correlation between the histamine H_2-receptor agonist activity and either pK_A values or relative molecular size as indicated by log MV values. Certain conclusions may be reached as to the structure-activity relationship. All the imidazolidine derivatives with 2,6-substitution in the phenyl ring are active as histamine H_2-receptor agonists; such substitution probably leads to an enhancement of activity by a mechanism other than alteration in physicochemical parameters (log P, pK_A, log MV) since these values vary widely within the series of 2,6-substituted derivatives. Substitution in the phenyl ring in the 3, 4 or 5 positions leads to a marked loss in histamine H_2-receptor agonist activity. The present findings indicate that there is sufficient stereochemical similarity between the conformations of the 2,6-disubstituted phenyliminoimidazolidine molecules and the active form of histamine at H_2-receptors to enable these compounds to activate histamine H_2-receptors. Durant, Ganellin & Parsons (1975) have defined the functional requirements of agonists at histamine H_1- and H_2-receptors. The important features in the histamine molecule for activation of H_2-receptors were considered to be the tautomeric property of the N–H group of the imidazole ring and the side chain containing the cationic N–H group. Durant *et al.* (1975) considered that this arrangement might allow a proton transfer to occur between the histamine molecule and the receptor site. The possibility for tautomerism exists in the imidazolidine moiety and it is possible that the interaction of these compounds with the H_2-receptor might enable a proton transfer process to occur. It has been shown that the ortho chlorine atoms of clonidine impose a conformational restriction on the molecule such that the minimum energy is found for a non-perpendicular structure, which enables the imidazolidine moiety to be conjugated with the phenyl moiety (Meerman-van Bentham, van der Meer, Mulder, Timmermans & van Zwieten, 1975); lack of substitution in the ortho positions would presumably lead to free rotation about the carbon-imino nitrogen bond and hence a more flexible molecule. Loss of activity at histamine H_2-receptors may thus occur, either through steric hindrance or as a result of a reduction in the proportion of molecules with the preferred conformation for histamine H_2-receptor agonist activity.

Table 2 includes data by Timmermans & van Zwieten (1977a) for the hypotensive activity in the anaesthetized, normotensive rat, of nine of the imidazolidine derivatives which were also used in the present study. It can be seen from Table 2 that only four of these imidazolidines which have been shown to decrease blood pressure and heart rate in the rat also activate guinea-pig atrial histamine H_2-receptors. Indeed all substituted phenyliminoimidazolidines appear to exert similar cardiovascular effects provided their lipophilicity allows penetration into the central nervous system (see Introduction for references). On the basis of these different structure-activity requirements for histamine H_2-receptor agonist activity and for hypotensive activity it seems unlikely, as some workers have suggested (see Introduction for references) that the imidazolidine derivatives reduce blood pressure and heart rate by activation of central histamine H_2-receptors. A similar conclusion was reached in a study in which the effects of clonidine, guanafacine and three structural analogues of clonidine were assessed on histamine H_2-receptor mediated increases in gastric acid secretion in the anaesthetized rat, and on heart rate and blood pressure (Medgett & McCulloch, 1979).

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References

ARUNLAKSHANA, O. & SCHILD, H.O. (1959). Some quantitative uses of drug antagonists. *Br. J. Pharmac. Chemother.* **14**, 48–58.

BLACK, J.W., DUNCAN, W.A.M., DURANT, G.J., GANELLIN, C.R. & PARSONS, M.E. (1972). Definition and antagonism of histamine H_2-receptors. *Nature, Lond.* **236**, 385–390.

BONDI, A. (1964). van der Waal's volumes and radii. *J. phys. Chem.* **68**, 441–450.

BRIMBELCOMBE, R.W., DUNCAN, W.A.M., DURANT, G.J., GANELLIN, C.R., PARSONS, M.E. & BLACK, J.W. (1975). The pharmacology of cimetidine, a new histamine H_2-receptor antagonist. *Br. J. Pharmac.*, **53**, 435–436P.

CSONGRADY, A. & KOBINGER, W. (1974). Investigations into the positive inotropic effect of clonidine in isolated hearts. *Naunyn-Schmiedebergs Arch. Pharmac.*, **282**, 123–128.

DURANT, G.J., GANELLIN, C.R. & PARSONS, M.E. (1975).

Chemical differentiation of histamine H_1- and H_2-receptor agonists. *J. med. Chem.*, **18**, 905–909.

FINCH, L., HARVEY, C.A., HICKS, P.E. & OWEN, D.A.A. (1977). Interaction between histamine H_2-receptor antagonists and the hypotensive effect of clonidine in rats. *Br. J. Pharmac.*, **59**, 477P.

FURCHGOTT, R.F. (1967). The pharmacological differentiation of adrenergic receptors. *Ann. N.Y. Acad. Sci.*, **139**, 553–570.

HANSCH, C. (1973). Quantitative approaches to pharmacological structure-activity relationships. In *International Encyclopedia of Pharmacology and Therapeutics*, Vol. 1, ed Cavallito C., pp. 75–165. Oxford: Pergamon Press.

HOEFKE, W. & KOBINGER, W. (1966). Pharmakologische Wirkungen des 2-(2,6-Dichlorophenylamino)-2-Imidazolinhydrochlorids, einer neuen, antihypertensiven Substanz. *Arzneimittelforsch (Drug Res.)*, **16**, 1038–1050.

HOEFKE, W., KOBINGER, W. & WALLAND, A. (1975). Relationship between activity and structure in derivatives of clonidine. *Arzneimittelforsch (Drug Res.)*, **25**, 786–793.

JAMES, T.N., BEAR, E.S., LANG, K.F. & GREEN, E.W. (1968). Evidence for adrenergic alpha receptor depressant activity in the heart. *Am. J. Physiol.*, **215**, 1366–1375.

JEN, T., VAN HOEVEN, H., GROVES, W., MCLEAN, C.A. & LOEV, D. (1975). Amidines and related compounds. 6. Studies on structure-activity relationships of antihypertensive and antisecretory agents related to clonidine. *J. med. Chem.*, **18**, 90–99.

KARPANNEN, H., PAAKKARI, I., PAAKKARI, P., HUOTARI, R. & ORMA, A.C. (1976). Possible involvement of histamine H_2-receptors in the hypotensive effect of clonidine. *Nature, Lond.* **359**, 587–588.

KOBINGER, W. (1978). Central α-adrenergic systems as targets for hypotensive drugs. *Rev. Physiol. Biochem. Pharmac.*, **81**, 39–100.

MCCULLOCH, M.W. & MEDGETT, I.C. (1979). Comparison of structure-activity requirements of clonidine analogues at prejunctional and postjunctional α-adrenoreceptors in guinea-pig isolated cardiovascular tissues. *Proc. Aust. Physiol. Pharmac. Soc.*, **10**, 125P.

MCCULLOCH, M.W., MEDGETT, I.C. & RAND, M.J. (1979). Effects of the histamine H_2-receptor blocking drugs burimamide and cimetidine on noradrenergic transmission in the isolated aorta of the rabbit and atria of the guinea-pig. *Br. J. Pharmac.*, **67**, 535–544.

MEDGETT, I.C. & MCCULLOCH, M.W. (1978). Structure-activity relationships of clonidine analogues at histamine H_2-receptors. *Proc. Aust. Physiol. Pharmac. Soc.*, **9**, 167P.

MEDGETT, I.C. & MCCULLOCH, M.W. (1979). Effects of clonidine, guanfacine and three imidazolidine derivatives related to clonidine on blood pressure, heart rate and gastric acid secretion in the anaesthetized rat. *Archs. int. Pharmacodyn.*, **240**, 158–168.

MEDGETT, I.C., MCCULLOCH, M.W. & STORY, D.F. (1979). Quantitative structure-activity relationships of clonidine analogues at postjunctional α-adrenoreceptors. *Proc. Aust. Physiol. Pharmac. Soc.* **10**, 124P.

MEERMAN-VAN BENTHAM, C.M. VAN DER MEER, K., MULDER, J.J.L., TIMMERMANS, P.B.M.W.M. & VAN ZWIETEN, P.A. (1975). Clonidine base: evidence for conjugation of both ring systems. *Molec. Pharmac.*, **11**, 667–670.

PAAKKARI, I., PAAKKARI, P. & KARPPANNEN, H. (1976). Antagonism of the central hypotensive effect of clonidine by the histamine H_2-receptor blocking agent metiamide. *Acta Physiol. Scand.*, **supp. 440**, 105.

SANDERS, J., MILLER, D.D. & PATIL, P.N. (1975). Alpha adrenergic and histaminergic effects of tolazoline-like imidazolidines. *J. Pharmac. exp. Ther.*, **195**, 362–371.

SCHMITT, P. (1977). The pharmacology of clonidine and related products. In *Antihypertensive Agents.* ed. Gross, F. *Handbook of Experimental Pharmacology*, XXXIX, pp. 299–396. Berlin, Heidelberg, New York: Springer.

STEINBERG, M.I. & HOLLAND, D.R. (1975). Separate receptors mediating the positive inotropic and chronotropic effect of histamine in guinea-pig atria. *Eur. J. Pharmac.*, **34**, 95–104.

STRUYKER BOUDIER, H., DE BOER, J., SMEETS, G., LIEN, E.J. & VAN ROSSUM, J. (1975). Structure-activity relationships for central and peripheral alpha adrenergic activities of imidazolidine derivatives. *Life Sci., Oxford* **17**, 377–386.

STRUYKER BOUDIER, H., SMEETS, G., BROUWER, G. & VAN ROSSUM, J. (1974). Central and peripheral alpha adrenergic activity of imidazoline derivatives. *Life Sci., Oxford*, **15**, 887–899.

STRUYKER BOUDIER, H., TEPPEMA, L., COOLS, A. & VAN ROSSUM, J. (1975). (3,4-Dihydroxy-phenylamino)-2-imidazoline (DPI), a new potent agonist at dopamine receptors mediating neuronal inhibition. *J. Pharm. Pharmac.*, **27**, 882–883.

TIMMERMANS, P.B.M.W.M. & VAN ZWIETEN, P.A. (1977a). Hypotensive and bradycardic activities of clonidine and related imidazolidines: structure-activity relationships. *Archs. int. Pharmacodyn.*, **228**, 237–250.

TIMMERMANS, P.B.M.W.M. & VAN ZWIETEN, P.A. (1977b). Central hypotensive and antihypertensive activities of imidazolidines, structurally related to clonidine. *Archs int. Pharmacodyn.*, **228**, 251–267.

TIMMERMANS, P.B.M.W.M. & VAN ZWIETEN, P.A. (1977c). Central and peripheral α-adrenergic effects of some imidazolidines. *Eur. J. Pharmac.*, **45**, 229–236.

VAN ZWIETEN, P.A. (1977). Antihypertensive action of clonidine (catapresan®) *Contr. Nephrol.*, **8**, 200–212.

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