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STRUCTURE-ACTIVITY RELATIONSHIPS OF CLONIDINE- AND TOLAZOLINE-LIKE COMPOUNDS AT HISTAMINE AND α-ADRENOCEPTOR SITES

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1 Thirty clonidine- and tolazoline-like compounds with differing phenyl ring substituents were tested for agonistic actions at histamine H_1-receptors (guinea-pig ileum), histamine H_2-receptors (guinea-pig driven right ventricular strips), post-junctional α-adrenoceptors (rat desheathed vas deferens) and pre-junctional α-adrenoceptors (inhibition of sympathetic stimulation in guinea-pig driven left atria).

2 All compounds were inactive at histamine H_1-receptors, while 21 of the 30 compounds displayed varying stimulant activity at H_2-receptors.

3 At post-junctional α-receptors all 30 compounds produced stimulant actions, whereas at pre-junctional α-receptors the compounds displayed either agonistic or antagonistic actions.

4 Thus structure-activity relationships (SAR) could only be validated for histamine H_2- and post-junctional α-receptor effects. These studies show that the most potent compounds are those with 2,6-phenyl substituents in which rotation is restricted so that the two rings are aplanar. Electronic effects of the substituents have a greater influence on activity at H_2- than at α-receptors.

5 The major difference in SAR involves the influence of substituents in the 3, 4 or 5 positions on the phenyl ring. The presence of these substituents abolish significant activity at H_2-receptors, while α-receptor stimulant activity is retained.

Introduction

Clonidine is an imidazolidine derivative that has been shown to produce both agonistic and antagonistic actions at pre- and post-junctional α-adrenoceptor sites (see Starke, 1977; Kobinger, 1978 for reviews; Medgett, McCulloch & Rand, 1978) and agonistic actions at histamine H_2-receptors (Karppanen & Westermann, 1973; Csongrady & Kobinger, 1974; Paakkari, Paakkari & Karppanen, 1976; Medgett & McCulloch, 1979). A similar spectrum of activity has also been noted with the imidazolines, tolazoline and tetrahydrozoline (Black, 1975; Sanders, Miller & Patil, 1975; Yellin, Sperow & Buck, 1975; Buck, Katchen, Lavenhar, Nelson & Yellin, 1978). However, others, for example oxymetazoline and naphazoline, are devoid of H_2-receptor stimulant activity (Sanders *et al.*, 1975).

Comparisons of structure-activity relationships of such molecules at histamine H_1- and H_2-receptors and at pre- and post-junctional α-receptors have not been detailed previously. In the present study 24 imidazolidines and 6 imidazolines have been assessed for actions at the above receptors in order to compare and contrast the molecular requirements necessary for agonistic activity. Contractile activity in guinea-pig ileal preparations was used to monitor H_1-receptor-mediated effects and positive inotropic activity in guinea-pig right ventricular strips to assess H_2-receptor actions. Contractile activity in desheathed rat vas deferens preparations was used to assess post-junctional α-receptor-mediated effects; modulation of responses to sympathetic stimulation in guinea-pig left atria was used to monitor pre-junctional α-receptor activity.

Methods

General

In all preparations studied the tissues were suspended under a 1 g tension and bathed in Krebs solution (NaCl 6.9, KCl 0.4, $MgSO_4$ 0.14, dextrose 2.0, $NaHCO_3$ 2.1, NaH_2PO_4 0.14, $CaCl_2$ 0.28 g/l, ascorbic acid 20 mg/l) maintained at 37°C (except ileum,

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32°C) aerated with 5% CO_2 in O_2. Responses of the guinea-pig right ventricular strip, left atrium and rat vas deferens were monitored isometrically with a Grass FT03c transducer coupled to a Grass 79D polygraph, while an isotonic frontal writing lever and a smoked drum were used for assessment of activity in ileal preparations. All compounds were investigated in concentrations up to 0.1 mmol/l.

Guinea-pig ventricle strip

Histamine H_2-receptor-mediated positive inotropic actions were monitored on guinea-pig right ventricular strips (Verma & McNeill, 1977; Kearney, Malta & Raper, 1979). The preparations were mounted on a platinum electrode and stimulated at 1 Hz with square wave pulses of 2.5 ms duration and twice the threshold voltage required to initiate contractile activity. The bathing solution contained propranolol and mepyramine (both 1 μmol/l) to preclude possible interference from β- and H_1-receptor mediated effects. Preliminary experiments with phentolamine (30 μmol/l) indicated that α-receptor-mediated effects did not interfere with the inotropic actions.

In each experiment, constant cumulative concentration-effect curves to histamine were first obtained at 20 min intervals and thereafter a curve to a test compound was established. Responses to the test compound were expressed as a percentage of the maximal response to histamine.

Compounds with intrinsic activities of less than 0.6 (histamine = 1) were tested for antagonistic actions by the method of Malta & Raper (1974); briefly, the concentration of the drug producing its maximal response was incubated with the tissue for 20 min and thereafter a histamine curve was superimposed. By this method, antagonism is assessed from shifts in the superimposed concentration-effect curves to histamine.

Ileal preparations

Histamine H_1-receptor activity was assessed in guinea-pig isolated ileal preparations obtained from reserpine-treated animals (2.5 mg/kg, 24 h previously). The bathing solution contained metiamide (50 μmol/l), propranolol (1 μmol/l), phentolamine (1 μmol/l) and desipramine (0.5 μmol/l) to prevent interference from actions of test compounds at other sites. Constant submaximal responses (40 to 60% E_{max}) to histamine were first obtained at 3 min intervals and thereafter the effects of the test drugs were monitored.

Guinea-pig left atria

Prejunctional α-receptor-mediated actions of the compounds were assessed by quantitating the reduction in inotropic response to field stimulation of the intramural sympathetic nerves in atropinized guinea-pig left atria. The preparations were driven at a frequency of 2 Hz with pulses of 1 ms duration and twice the threshold voltage required for initiation of contractions. A 10 fold increase in this voltage for a period of 60 s produced maximal positive inotropic actions which were due to the activation of intramural sympathetic nerves. Inotropic responses were abolished in the presence of either tetrodotoxin (10 ng/ml) or propranolol (1 μmol/l). Responses to sympathetic stimulation were elicited at 15 min intervals.

Preliminary experiments showed that the magnitude of the evoked responses remained constant over a 2 h period even though resting contractility tended to decline. Prejunctional α-receptor mediated actions of the compounds were assessed from alterations in the evoked sympathetic responses obtained in the presence of mepyramine (1 μmol/l), cocaine (10 μmol/l) and atropine (1 μmol/l). Prejunctional H_2-receptors do not appear to influence sympathetic responses in this preparation, since the H_2-receptor selective agonist, dimaprit ($\leqslant$0.1 mmol/l), did not affect responses to sympathetic stimulation. The H_2-receptor antagonists, cimetidine and metiamide (10 μmol/l), produced a small enhancement of the evoked responses, possibly due to weak prejunctional α-receptor antagonistic actions (Griffith, Marshall & Nasmyth, 1978). For this reason they were not included in the bathing solution.

In each experiment two control responses to sympathetic stimulation were first obtained and then increasing cumulative concentrations of the drug under test were added to the bathing solution 5 min before the next stimulation period. The tissue was not washed during the course of the experiment. The results were calculated as a percentage change in evoked inotropic activity.

Rat vas deferens

Post-junctional α-receptor activity was monitored in desheathed rat vas deferens preparations obtained from reserpine-treated animals (1 mg/kg, 24 h previously). Contractile activity was assessed in the presence of desipramine (0.5 μmol/l), propranolol, mepyramine, atropine (all 1 μmol/l) and metiamide or cimetidine (10 μmol/l). Corticosterone at a concentration of 50 μmol/l did not affect the response to noradrenaline and was therefore not included in the bathing solution. Desipramine (0.5 μmol/l) was used in preference to cocaine to block the neuronal uptake process, since the latter agent has been shown to produce a sensitization of the post-junctional α-receptors in this tissue (Warming, Pennefather & Handberg, 1978).

Dose-response curves to increasing concentrations

of noradrenaline and the drugs under test were established using a 5 min cycle and a drug contact time sufficient to allow maximum responses (<30 s). In each experiment two curves to noradrenaline were obtained at 20 min intervals and thereafter a curve was established to the drug under test. Results were expressed as a percentage of the maximal response to noradrenaline.

The drugs used were cimetidine, metiamide and dimaprit (Smith, Kline & French); reserpine, desipramine, phentolamine, naphazoline, xylometazoline and tolazoline (Ciba-Geigy); oxymetazoline (Glaxo-Allenbury); mepyramine (May & Baker); atropine, noradrenaline, yohimbine and histamine (Sigma); propranolol (Imperial Chemical Industries); prazosin (Pfizer); thymoxamine (Warner); cocaine (Macfarlan Smith) and the St compounds listed in Table 1 (Boehringer Ingelheim).

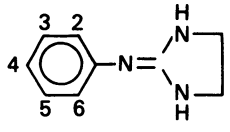
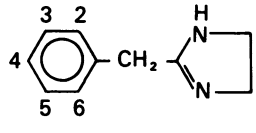
Stock solutions of the compounds (10 mmol/l) were dissolved in 10 mmol/l HCl and suitable dilutions made up in Krebs solution containing 20 mg/l ascorbic acid.

Results

Guinea-pig ventricle strips

Histamine produced positive inotropic responses in the ventricular preparations, a five fold increase in tension being obtained with maximal concentrations of the amine. The mean EC_{50} concentration for this action of histamine was 1.06 ± 0.88 μmol/l (n = 135). Of the 24 imidazolidine and 6 imidazoline derivatives tested, 18/24 and 3/6 compounds respectively, produced positive inotropic actions which were competitively antagonized by the H_2-receptor antagonists metiamide or cimetidine (10 μmol/l). The concentration–effect curves to the active compounds were generally parallel to those of histamine. Traces from

Table 1 Structures of the imidazolidines and imidazolines used in the present study

Drug		*Ring substitution*	*Drug*		*Ring substitution*
Imidazolidines					
*Clonidine	(HCl)	2,6-diCl	*St375	(HNO_3)	2-Cl, 4-CH_3
*St464	(HCl)	2,6-diBr	*St476	(HCl)	2,3-diCl
*St1912	(base)	2,6-diF	St608	(HCl)	2-Cl, 3-CH_3
*St1923	(HCl)	2-Cl, 6-F	Tramazoline	(HCl)	2,3-cyclohexano
*St93	(HCl)	2-Cl, 6-CH_3	*St96	(base)	2-Cl
*St95	(HCl)	2,6-diCH_3	St681	(HNO_3)	2-F
St1697	(base)	2-CH_3, 6-CH_2CH_3	St371	(HCl)	2-CF_3
*St91	(HCl)	2,6-diCH_2CH_3	*St732	(HCl)	2,4,6-triCl
*St475	(HCl)	2,5-diCl	St666	(HBr)	2,6-diCl, 4-OH
St585	(HCl)	2-CH_3, 5-Cl	*St89	(HCl)	2,4,6-triCH_3
St600	(HCl)	2-CH_3, 5-F	St1943	(HBr)	3,4-diOH
*St363	(HCl)	2,4-diCl	*St465	(base)	unsubstituted
Imidazolines					
St1913	(HCl)	2,6-diCl			
Naphazoline	(HCl)	2,3-benzo			
St71	(HCl)	2,4,6-triCH_3			
Xylometazoline	(HCl)	2,6-diCH_3, 4-$C(CH_3)_3$			
Oxymetazoline	(HCl)	2,6-diCH_3, 3-OH, 4-$C(CH_3)_3$			
Tolazoline	(HCl)	unsubstituted			

* Compounds marked with an asterisk were used in physicochemical correlations (see Discussion).

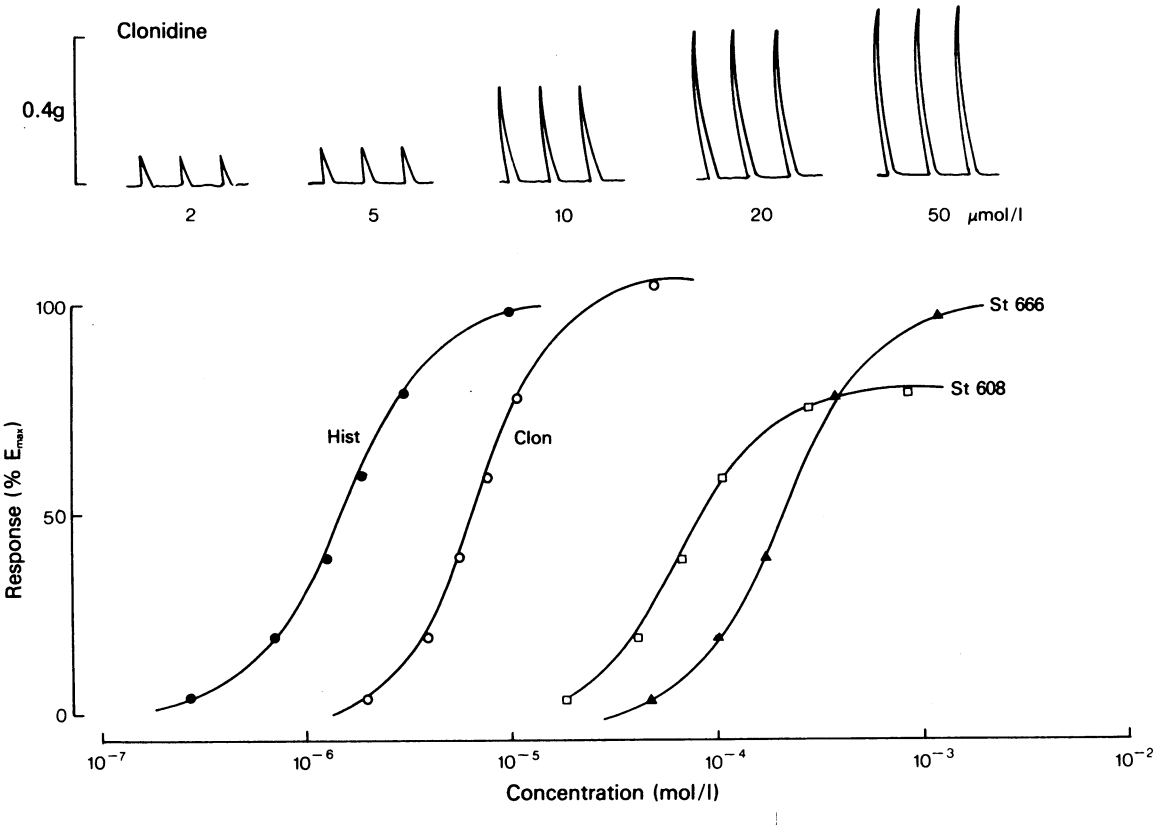


Figure 1 Traces showing the positive inotropic response to cumulative concentrations of clonidine (Clon) in a guinea-pig driven right ventricular strip preparation (1 Hz). The graphs show mean concentration-effect curves to histamine (Hist) and three of the imidazolidines tested ($n \geqslant 4$ comparisons). Results are expressed in terms of the maximal response to histamine (E_{max}) and individual points show mean concentrations for a given percentage effect.

an experiment with clonidine and mean concentration-effect curves for a number of the compounds tested are shown in Figure 1.

Table 2 shows the intrinsic activities and pD_2 values of the compounds displaying agonistic effects. Activity ratios of the compounds with respect to histamine were calculated from individual experiments (drug EC_{50}:histamine EC_{50}) whereas those to clonidine were obtained from the antilog of the difference in mean pD_2 values.

Compounds that were inactive (tramazoline, St363, St476, St585, St732, St1943, St71, xylometazoline, oxymetazoline) as positive inotropic agents and those which possessed intrinsic activities of less than 0.6 (histamine = 1.0) did not display any antagonistic activity towards histamine.

Ileal preparations

All compounds, with the exception of St732, failed to produce H_1-receptor mediated contractions in guinea-pig ileal preparations. St732, a compound that was found to be inactive at H_2-receptor sites, produced threshold responses at 0.1 mmol/l which were antagonized by mepyramine (1 μmol/l). None of the agents studied possessed H_1-receptor antagonistic actions since concentrations up to 0.1 mmol/l failed to inhibit subsequent responses to histamine.

Guinea-pig left atria

Clonidine (10 nmol/l to 1 μmol/l) produced a concentration-related inhibition of the inotropic response to sympathetic nerve stimulation (mean $EC_{50} = 0.13 \pm 0.03$ μmol/l), the mean maximal reduction being $56 \pm 4\%$ ($n = 4$). The inhibition produced by clonidine was reversed by phentolamine (5 μmol/l) and yohimbine (0.1 μmol/l), and was antagonized by the prior addition of these agents to the bathing solution. Thymoxamine and prazosin (10

μmol/l) were without effect on responses to clonidine. The latter antagonists were also without effect on inotropic responses to sympathetic stimulation whereas phentolamine and yohimbine (10 nmol/l to 5 μmol/l) produced an enhancement of the inotropic responses to sympathetic stimulation, mean maximal increases in tension of 56 ± 10% (n = 3) and 60 ± 5% (n = 4) above control values being obtained respectively. The receptor involved thus appears to be similar to pre-junctional α-receptors described in other tissues (see Starke, 1977 for review; Drew, 1976, 1977; Doxey, Smith & Walker, 1977; Doxey, 1979). Reduction of sympathetically-induced increases in inotropic activity in the guinea-pig left atria thus provides a suitable measure for establishing full concentration–effect curves for drugs at pre-junctional α-receptor sites. One limitation of this method occurs when the drugs exert post-junctional β-receptor-mediated effects, a situation which does not arise with the agents used in the present study. Of all compounds examined, 10 imidazolidine and one imidazoline derivative (oxymetazoline) produced inhibition of sympathetic responses. These inhibitory responses were reversed by the addition of phentolamine (5 to 50 μmol/l) to the bath. Table 3 shows the mean pD_2 values (negative logarithm of the concentration producing 50% of an individual compound's inhibitory effect), maximal percentage reduction of the responses and the activity ratios (antilog [pD_2 clonidine − pD_2 drug]) for the active compounds. Noradrenaline could not be tested since this amine produces post-junctional activation of β-receptors thereby interfering with quantitation of the response.

The compounds St71, St89, St363, St465, St475, St476, St600, St681 and St1913 produced concentration-dependent *increases* in the responses to nerve stimulation which were of similar magnitude to those produced by phentolamine and yohimbine. The enhanced responses produced with these compounds were reversed by clonidine (10 μmol/l).

Concentrations of 10 nmol/l to 50 μmol/l of the remaining compounds produced only small and inconsistent effects on the responses to sympathetic stimulation. Under the present conditions it is impossible to determine whether the absence of effect of these agents is due to lack of affinity for the pre-junctional α-adrenoceptors or to complications resulting from dualist activity.

Table 2 Histamine H_2-receptor actions of imidazolidines and imidazolines

Drug	*Activity ratio (Histamine)*	*Activity ratio (Clonidine)*	α	pD_2
Histamine	1.00	0.03	1.00	5.80 (0.05)
Imidazolidines				
Clonidine	5.36 (0.35)	1.00	1.07 (0.05)	5.25 (0.15)
St93	6.48 (1.21)	1.35	0.98 (0.03)	5.12 (0.04)
St464	6.87 (1.59)	1.58	1.04 (0.05)	5.05 (0.22)
St1912	12.6 (1.30)	1.86	1.02 (0.02)	4.98 (0.09)
St1923	18.4 (2.6)	2.63	1.00 (0.06)	4.83 (0.08)
St95	21.3 (4.3)	1.74	0.84 (0.10)	5.01 (0.08)
St1697	28.8 (4.1)	6.76	1.00 (0.10)	4.42 (0.07)
St91	30.8 (5.2)	6.46	1.00 (0.04)	4.44 (0.06)
St681	72.0 (9.9)	10.97	0.98 (0.02)	4.21 (0.12)
St608	87.6 (23.1)	8.91	0.75 (0.04)	4.30 (0.16)
St96	87.6 (15.6)	13.49	0.89 (0.10)	4.12 (0.08)
St89	96.7 (12.6)	7.59	0.77 (0.16)	4.37 (0.07)
St371	167.8 (12.8)	17.78	0.69 (0.32)	4.00 (0.19)
St375	182.2 (28.4)	13.49	0.32 (0.02)	4.12 (0.18)
St465	191.7 (22.4)	27.54	1.08 (0.08)	3.81 (0.09)
St600	206.0 (43.0)	16.60	0.76 (0.12)	4.03 (0.11)
St475	244.4 (36.9)	19.06	0.40 (0.09)	3.97 (0.14)
St666	252.4 (22)	38.02	1.02 (0.05)	3.67 (0.05)
Imidazolines				
St1913	5.45 (0.75)	0.95	1.09 (0.06)	5.27 (0.06)
Tolazoline	21.7 (2.7)	6.92	1.09 (0.04)	4.41 (0.10)
Naphazoline	217.2 (12.3)	22.39	0.27 (0.05)	3.90 (0.07)

Mean activity ratios with respect to histamine (drug EC_{50}: histamine EC_{50}) and clonidine (antilog [pD_2 clonidine − pD_2 drug]), intrinsic activities (α, histamine = 1) and pD_2 values are shown. Figures in parentheses indicate s.e. mean obtained from at least 4 experiments.

Rat vas deferens

Noradrenaline produced concentration-related contractions of the desheathed rat vas deferens preparation (mean $EC_{50} = 0.42 \pm 0.07$ μmol/l, $n = 127$).

In contrast to the findings in the other preparations used, all the imidazolidines and imidazolines tested induced concentration-related contractions of the vas deferens which were abolished by phentolamine (5 μmol/l). Figure 2 shows traces from an experiment using clonidine and mean concentration–effect curves for some of the compounds tested. Table 4 shows the mean activity ratios (drug EC_{50}:noradrenaline EC_{50} and antilog [pD_2 clonidine − pD_2 drug), intrinsic activities (α, noradrenaline = 1.00) and the pD_2 values of the compounds. The drugs ranged in activity from equiactive, to 100 times less active than noradrenaline and all had intrinsic activities of less than unity.

Assessment of possible antagonistic actions of the compounds was attempted; however, as noted by Mujić & van Rossum (1965), prolonged contact of the tissue with these agents induces either desensitization and/or spontaneous activity such that it is not possible to establish reliable superimposed responses to noradrenaline.

Discussion

The results of the present experiments with imidazolidine and imidazoline derivatives indicate that the compounds lack affinity for histamine H_1-receptors while many have agonistic actions at histamine H_2-receptor sites. While all the compounds tested displayed agonistic effects at post-junctional α-receptors, evaluation of their actions at pre-junctional sites was complicated by the fact that both agonistic and antagonistic effects were obtained. Discussion of structure activity relationships for agonistic actions must therefore be confined to the effects of the compounds at histamine H_2- and post-junctional α-receptors.

At both histamine H_2-receptors and post-junctional α-receptors, molecules possessing a 2,6-disubstitution in the phenyl ring are the most active irrespective of whether the compound contains an imidazolidine or imidazoline ring. In the imidazolidine series, clonidine (2,6-dichloro), St464 (2,6-dibromo), St95 (2,6-dimethyl), St93 (2-chloro, 6-methyl), St1697 (2-methyl, 6-ethyl) and St91 (2,6-diethyl) possess substituents that are large enough to produce interactions which are energetically unfavourable with the nitrogen atoms in the heterocyclic ring. Therefore the most stable conformation is that in which the two rings are aplanar (but not necessarily at right angles to each other). The lower potency of the monosubstituted compounds (St96, monochloro and St681, monofluoro) may be a reflection of the greater degree of rotation which is possible in such molecules. The unsubstituted imidazolidine, St465, which on the basis of energy barriers would be expected to be a near planar molecule (Timmermans, van Zwieten, Meerman-van Benthem, van der Meer & Mulder, 1977b) has a very low activity at both histamine H_2- and post-junctional α-receptors.

In contrast to the unsubstituted imidazolidine,

Table 3 Inhibition of stimulation-induced transmitter release by imidazolidine and imidazoline derivatives

Drug	*Activity ratio (Clonidine)*	*Maximal % reduction*	pD_2
Imidazolidines			
Clonidine	1.00	56 (4)	6.93 (0.12)
St95	8.13	32 (7)	6.02 (0.11)
St91	8.13	45 (11)	6.02 (0.08)
St371	8.13	70 (3)	6.02 (0.53)
St1943	12.30	56 (16)	5.84 (0.20)
St93	19.06	70 (20)	5.65 (0.19)
St666	20.89	35 (15)	5.61 (0.09)
St96	54.95	65 (11)	5.19 (0.27)
St464	208.93	73 (2)	4.61 (0.08)
Tramazoline	323.59	95 (1)	4.42 (0.08)
Imidazolines			
Oxymetazoline	17.38	38 (9)	5.69 (0.11)

Mean activity ratios (antilog [pD_2 clonidine − pD_2 drug]), maximal percentage reductions of response to sympathetic stimulation and pD_2 values are shown. Figures in parentheses indicate s.e. mean from at least 4 experiments.

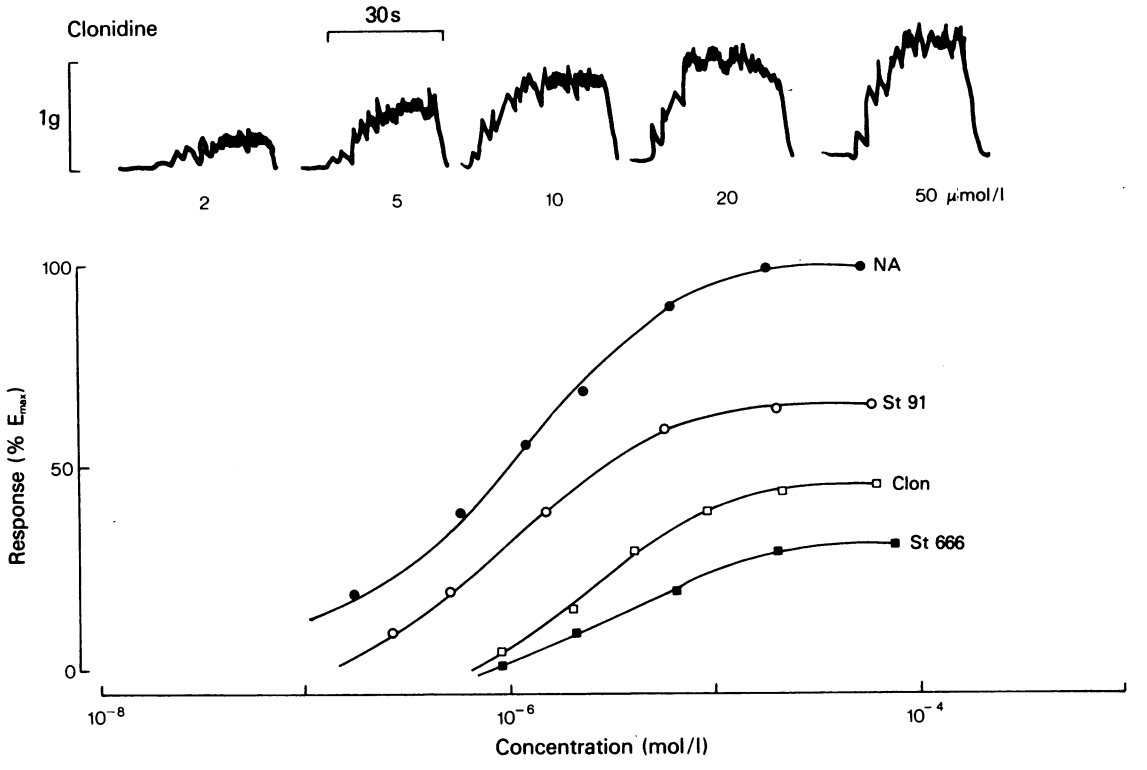


Figure 2 Traces showing contractions to individual concentrations of clonidine (Clon) in a desheathed vas deferens preparation from the rat. The graphs show mean concentration–effect curves ($n \geqslant 4$ comparisons) to noradrenaline (NA) and three of the imidazolidines studied. Responses are expressed in terms of the maximal response to noradrenaline (E_{max}) and individual points show mean concentrations for a given percentage effect.

St465, the imidazoline, tolazoline, shows high activity. However, in this molecule the orientation of the two rings and the two hydrogen atoms around the central 'bridging' carbon atom would be in a roughly tetrahedral arrangement, thus the phenyl and imidazoline rings could be in different planes, a factor which might explain its greater potency.

Consideration of the activities of the compounds at H_2-receptor sites indicate that within the series of 2,6-disubstituted imidazolidines, those which have halide substitutions (clonidine, St464, St1912) are more active than those with alkyl substitutions (St95, St1697, St91). This suggests that molecules having electron-withdrawing (e.g. chloro, bromo, fluoro) substituents have greater activity than those with electron-donating groups (e.g. methyl, ethyl). In contrast to the actions of the compounds at H_2-receptors, electronic effects appear to have little influence on activity at post-junctional α-receptor sites. Thus the 2,6-diethyl analogue, St91, and the 2-methyl, 6-ethyl compound, St1697, both of which may be expected to donate electrons to the phenyl ring are approximately equiactive to the dichloro compound, clonidine.

The introduction of substituents in addition to, or in other than the 2,6-positions of the phenyl ring, lead to marked differences in the activity of the compounds at histamine H_2- and post-junctional α-receptors. Molecules with varying dichloro (St476, St363, St475) or halo-alkyl (St375, St600, St585) and dihydroxy (St1943) substituents are weak or inactive as histamine H_2-receptor agonists. Compounds with three or more substituents on the phenyl ring as in the imidazolidines St89, St666, St732 and the imidazolines St71, xylometazoline and oxymetazoline, also lack affinity for histamine H_2-receptors.

In contrast, activity is retained at post-junctional α-receptors when the phenyl ring is substituted in any position, for example St476 (2,3-dichloro), St608 (2-chloro, 3-methyl), St363 (2,4-dichloro), St375 (2-chloro, 4-methyl), St600 (2-methyl, 5-fluoro), St475 (2,5-dichloro), St585 (2-methyl, 5-chloro), oxymetazoline, naphazoline and xylometazoline all range from

0.37 to 8.7 times less active than clonidine. In addition it does not appear that the nature of the substituent group (e.g. chloro or methyl) affects the activity.

These results suggest that steric hindrance through substitution in the 3-, 4- or 5-position may account for reduced activity of the compounds at H_2-receptor sites, while this factor assumes little importance in terms of post-junctional α-receptor actions. However it is possible that other molecular properties may be affected by substitutions in these positions and that these may account for the reduced activity at H_2-receptor sites.

Although steric effects alone may determine biological activity within the series of compounds studied, other physicochemical properties may also play an important role. Physicochemical data for 15 of the imidazolidines studied (Table 1) was available from the work of Timmermans, Brands & van Zwieten (1977a) and Timmermans & van Zwieten (1978). Linear, multiple and polynomial regression analyses were used to determine possible correlations between pD'_2 values (pD_2 values corrected for the degree of ionization at pH 7.4) with pKa, log P (octanol-phosphate buffer partition coefficient, pH 7.4) or $(\log P)^2$.

No correlation was found between pD'_2 and either pKa ($0.25 < P < 0.10$), log P, ($0.25 < P < 0.10$) or $(\log P)^2$ ($0.5 < P < 0.4$) at histamine H_2-receptors. A correlation between pKa and pD'_2 values for α-receptor activity just failed to reach statistical significance

Table 4 Postjunctional α-adrenergic receptor actions of imidazolidine and imidazoline derivatives

Drug	*Activity ratio (Noradrenaline)*	*Activity ratio (Clonidine)*	α	*pD_2*
(−)-Noradrenaline	1.00		1.00	6.47 (0.03)
Imidazolidines				
Tramazoline	1.70 (0.30)	0.11	0.62 (0.05)	6.52 (0.05)
St91	1.99 (0.24)	0.35	0.65 (0.04)	6.03 (0.06)
Clonidine	3.10 (0.77)	1.00	0.43 (0.03)	5.58 (0.08)
St1697	3.13 (0.79)	0.30	0.66 (0.07)	6.10 (0.12)
St1912	5.86 (1.15)	0.66	0.13 (0.01)	5.76 (0.15)
St464	6.41 (1.05)	0.50	0.23 (0.04)	5.88 (0.03)
St1923	7.52 (1.73)	0.76	0.28 (0.01)	5.70 (0.06)
St475	8.63 (2.05)	0.60	0.44 (0.06)	5.80 (0.16)
St93	10.13 (1.25)	1.66	0.32 (0.03)	5.36 (0.09)
St89	10.45 (0.73)	2.24	0.20 (0.02)	5.23 (0.04)
St608	10.77 (2.12)	1.10	0.23 (0.03)	5.54 (0.13)
St585	10.77 (2.56)	1.20	0.42 (0.07)	5.50 (0.02)
St732	11.27 (1.01)	1.41	0.31 (0.01)	5.43 (0.04)
St476	13.53 (2.26)	1.26	0.31 (0.02)	5.48 (0.12)
St1943	13.94 (2.33)	2.14	0.67 (0.03)	5.25 (0.05)
St95	16.01 (4.34)	1.62	0.49 (0.04)	5.37 (0.15)
St666	17.96 (2.72)	1.91	0.38 (0.04)	5.30 (0.06)
St600	18.38 (3.37)	2.00	0.31 (0.04)	5.28 (0.22)
St363	23.61 (3.95)	2.29	0.27 (0.02)	5.22 (0.13)
St375	26.81 (2.83)	3.72	0.13 (0.01)	5.01 (0.05)
St681	31.50 (5.93)	2.51	0.15 (0.02)	5.18 (0.12)
St96	55.25 (6.64)	5.13	0.23 (0.04)	4.87 (0.02)
St371	75.78 (18.52)	6.46	0.23 (0.06)	4.77 (0.10)
St465	97.90 (21.61)	8.91	0.09 (0.01)	4.63 (0.07)
Imidazolines				
Oxymetazoline	1.16 (0.42)	0.18	0.48 (0.08)	6.33 (0.05)
Naphazoline	4.99 (0.90)	0.79	0.59 (0.06)	5.68 (0.06)
Xylometazoline	8.91 (0.56)	0.63	0.47 (0.04)	5.78 (0.28)
St71	17.80 (1.71)	2.34	0.34 (0.04)	5.21 (0.04)
St1913	18.35 (0.87)	2.40	0.25 (0.03)	5.20 (0.03)
Tolazoline	51.05 (10.79)	5.01	0.09 (0.01)	4.88 (0.14)

Mean activity ratios with respect to noradrenaline (drug EC_{50}: noradrenaline EC_{50}), and clonidine (antilog [pD_2 clonidine − pD_2 drug]), intrinsic activities (α, noradrenaline = 1.00) and pD_2 values are shown. Figures in parentheses indicate s.e. mean obtained from at least 4 experiments.

($0.1 < P < 0.05$); however, a significant correlation was obtained between pD'_2 and log P ($pD'_2 = 5.538 + 0.274$ (± 0.093) log P, $r = 0.634$, $s = 0.351$, $F = 8.754$, $0.02 < P < 0.01$). The latter result is in accord with previous findings by Timmermans *et al.* (1977a) and Medgett, McCulloch & Story (1979) who studied the actions of clonidine derivatives at 'central' α-receptors and in the guinea-pig aorta respectively. These results suggest that both steric and physicochemical properties may play a role in determining activity of imidazolines at α-receptor sites, whereas steric effects dominate the events for histamine H_2-mediated activity.

The relatively small number of compounds which display agonistic actions at pre-junctional α-receptors precludes detailed analysis of structure-activity relationships. However, in general terms it appears that 2,6-disubstituted compounds are the most active and that activity can be maintained in compounds with substitutions in other positions on the phenyl ring. These general results are in accord with those found for the actions of the compounds at post-junctional α-receptor sites; however, the relative potencies of the compounds with respect to clonidine differ to a marked extent at the two sites.

The actions of the imidazolidine and imidazoline derivatives at pre-junctional α-receptors are reminiscent of partial agonists in that the compounds produced varying degrees of inhibition of the responses to sympathetic nerve stimulation. It is of interest that molecules which possessed low values of intrinsic activity at post-junctional α-receptor sites in the rat vas deferens generally enhanced responses to nerve stimulation in the atria, while those which displayed relatively higher intrinsic activities generally inhibited responses to nerve stimulation. Partial agonistic effects at pre-junctional α-receptor sites have previously been demonstrated for oxymetazoline and clonidine (Medgett & McCulloch, 1978; Medgett *et al.*, 1978).

Although numerous workers have studied pre- and post-junctional α-receptor actions of various imidazolines and imidazolidines, only Timmermans & van Zwieten (1977) have tested and developed structure-activity relationships using a comparable number of compounds to those tested in the present experiments. These workers assessed central α-adrenoceptor actions in terms of decreases in blood pressure in anaesthetized rats. Structure–activity relationships were developed after generating equations to relate the fall in blood pressure to physicochemical properties of the molecules. They concluded that for potent activity, the phenyl and imidazoline rings should be aplanar and that the phenyl ring should contain 2,6-disubstituents, one substituent having a similar bulk to chlorine and the other of a sufficient size to maintain the aplanar conformation. In addition they suggested that the 4-position of the phenyl ring should remain unsubstituted.

Although there are similarities in the general conclusions reached by Timmermans & van Zwieten (1977) and those found in the present study, there are a number of differences. These may be related either to differences in the 'central' α-receptors and those found in the rat vas deferens, or in the methods used to generate the results. Thus for instance, in the rat vas deferens 2,3-, 2,4- and 2,5-dichloro (St476, 363 and 475) have a similar order of potency and are more active than the 2-chloro substituted compound (St96), while at central α-receptors these disubstituted compounds vary widely in potency compared to St96. In our results, the 2,3- and 2,5-disubstituted compounds have a comparable potency, and thus do not support the notion that only one side of the phenyl ring is of importance to α-receptor activity (Timmermans & van Zwieten, 1977). In addition, 4-substitution does not appear to be unfavourable to activity in the vas deferens as was found at central α-receptors (cf. present results with St89 and St95 with Tz10 and Tz20 of Timmermans & van Zwieten, 1977).

In summary, molecules with 2,6-disubstitutions, which assume an orientation where the phenyl and imidazoline or imidazolidine rings are in different planes, possess the greatest activity at both histamine H_2- and post-junctional α-receptor sites. The electronic effects of the substituents appear to have a greater influence on actions at H_2- rather than the α-receptors. This conformational requirement is similar to the proposed crystal structure of clonidine (Meerman-van Benthem, van der Meer, Mulder, Timmermans & van Zwieten, 1975) in which the ground state conformation of the molecule occurs when the two rings were aplanar.

The major differences in the SAR of the molecules at H_2- and α-receptor sites involves the influence of substitutions in the 3, 4 or 5 positions of the phenyl ring. Substitution in these positions preclude potent activity at H_2-receptor sites (possibly due to steric hindrance) while α-receptor activity is maintained.

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References

BLACK, J.W. (1975). Histamine receptors. *Proceedings of the VIth International Congress of Pharmacology*, ed. Tuomisto, J. & Paasonen, M.K. **1**, 3.

BUCK, S.H., KATCHEN, M.S., LAVENHAR, S.R., NELSON, E.G. & YELLIN, T.O. (1978). H_2-Histaminergic and α-adrenergic activity of d-tetrahydrozoline. *Fedn Proc.*, **37**, 393.

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

DOXEY, J.C. (1979). Pre- and post-synaptic effects of α-agonists in the anococcygeus muscle of the pithed rat. *Eur. J. Pharmac.*, **54**, 185–189.

DOXEY, J.C., SMITH, C.F.C. & WALKER, J.M. (1977). Selectivity of blocking agents for pre- and post-synaptic α-adrenoceptors. *Br. J. Pharmac.*, **60**, 91–96.

DREW, G.M. (1976). Effects of α-adrenoceptor agonists and antagonists on pre- and post-synaptically located α-adrenoceptors. *Eur. J. Pharmac.*, **36**, 313–320.

DREW, G.M. (1977). Pharmacological characterisation of the presynaptic α-adrenoceptor in the rat vas deferens. *Eur. J. Pharmac.*, **42**, 123–130.

GRIFFITH, O.R., MARSHALL, I. & NASMYTH, P.A. (1978). Blockade of pre-synaptic α-adrenoceptors by metiamide. *Br. J. Pharmac.*, **62**, 416P.

KARPPANEN, H.O. & WESTERMANN, E. (1973). Increased production of cyclic AMP in gastric tissue by stimulation of H_2-receptors. *Naunyn-Schmiedebergs Arch. Pharmac.*, **279**, 83–87.

KEARNEY, P.E., MALTA, E. & RAPER, C. (1979). Actions of some imidazole compounds on histamine H_2-receptors in guinea-pig ventricular strips. *Clin. exp. Pharmac. Physiol.*, **6**, 218.

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

MALTA, E. & RAPER, C. (1974). Non-catechol phenylethanolamines: agonistic and antagonistic actions on β-adrenoceptors in isolated tissues from the guinea-pig. *Clin. exp. Pharmac. Physiol.*, **1**, 259–268.

MEDGETT, I. & MCCULLOCH, M.W. (1978). Pre- and post-junctional effects of clonidine in isolated guinea-pig atria. *Clin. exp. Pharmac. Physiol.*, **5**, 253–254.

MEDGETT, I.C. & MCCULLOCH, M.W. (1979). Comparison of the α-adrenergic and H_2-histaminergic effects of clonidine [2-(2,6-dichlorophenylamino)-2-imidazole hydrochloride] and St476 [2-(2,3-dichlorophenylamino)-2-imidazole hydrochloride]. *Clin. exp. Pharmac. Physiol.*, **6**, 205.

MEDGETT, I.C., MCCULLOCH, M.W. & RAND, M.J. (1978). Partial agonist action of clonidine on prejunctional and post-junctional α-adrenoceptors. *Naunyn-Schmiedebergs Arch. Pharmac.*, **304**, 215–221.

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

MUJIĆ, M. & VAN ROSSUM, J.M. (1965). Comparative pharmacodynamics of sympathomimetic imidazolines: studies on intestinal smooth muscle of the rabbit and the cardiovascular system of the cat. *Archs int. Pharmacodyn.*, **155**, 432–449.

PAAKKARI, I., PAAKKARI, P. & KARPPANEN, H. (1976). Antagonism of the central hypotensive effect of clonidine by the histamine H_2-blocking agent, metiamide. *Acta physiol. scand. Suppl.* **440**, 152.

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

STARKE, K. (1977). Regulation of noradrenaline release by presynpatic receptor systems. *Rev. Physiol. Biochem. Pharmac.*, **77**, 1–124.

TIMMERMANS, P.B.M.W.M., BRANDS, A. & VAN ZWIETEN, P.A. (1977a). Lipophilicity and brain disposition of clonidine and structurally related imidazolidines. *Naunyn-Schmiedebergs Arch. Pharmac.*, **300**, 217–226.

TIMMERMANS, P.B.M.W.M. & VAN ZWIETEN, P.A. (1977). Quantitative structure-activity relationships in centrally acting imidazolidines structurally related to clonidine. *J. med. Chem.*, **20**, 1636–1644.

TIMMERMANS, P.B.M.W.M. & VAN ZWIETEN, P.A. (1978). Dissociation constants of clonidine and structurally related imidazolidines. *Arzneimittel Forsch.*, **28**, 1676–1681.

TIMMERMANS, P.B.M.W.M., VAN ZWIETEN, P.A., MEERMAN-VAN BENTHEM, C.M., VAN DER MEER, K. & MULDER, J.J.C. (1977b). Quantum chemical studies on clonidine and related derivatives. *Arzneimittel Forsch.*, **27**, 2266–2270.

VERMA, S.C. & MCNEILL, J.H. (1977). Cardiac histamine receptors: differences between left and right atria and ventricle. *J. Pharmac. exp. Ther.*, **200**, 352–362.

WARMING, S.E., PENNEFATHER, J.N. & HANDBERG, G.M. (1978). The actions of noradrenaline at pre-junctional adrenoceptors in the rat vas deferens in the presence of neuronal uptake inhibitors. *Clin. exp. Pharmac. Physiol.* In press.

YELLIN, T.O., SPEROW, J.W. & BUCK, S.H. (1975). Antagonism of tolazoline by histamine H_2-receptor blockers. *Nature, Lond.*, **253**, 561–563.

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