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PRE- AND POSTJUNCTIONAL EFFECTS OF CLONIDINE- AND OXYMETAZOLINE-LIKE COMPOUNDS IN GUINEA-PIG ILEAL PREPARATIONS

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1 Noradrenaline and 28 imidazolidine (clonidine-like) and imidazoline (oxymetazoline-like) compounds with various phenyl ring substituents have been examined for their ability to inhibit responses to transmural stimulation and exogenous acetylcholine in ileal preparations from reserpine-treated guinea-pigs.

2 The bathing solution contained propranolol, mepyramine, cimetidine and desipramine to preclude interference with the responses by other than the α-receptor-mediated actions of the compounds.

3 In transmurally stimulated preparations the inhibitory response to noradrenaline is due to a combination of prejunctional α-adrenoceptor stimulation and a postjunctional depressant effect that does not involve adrenoceptor activation.

4 Of the 28 imidazolidines and imidazolines studied, 21 inhibited transmurally elicited responses. In the various compounds studied this effect involved actions at pre- or postjunctional sites as indicated by (a) the frequency-dependence of the inhibitory response, (b) its susceptibility to blockade by α-receptor antagonists and (c) the relative concentrations required to inhibit responses to transmural stimulation and exogenous acetylcholine.

Introduction

Catecholamines such as noradrenaline, adrenaline and isoprenaline, produce an adrenoceptor-mediated inhibition of responses to transmural stimulation in guinea-pig ileal preparations (Paton & Vizi, 1969; Kosterlitz, Lydon & Watt, 1970). It is now well established that in this tissue, α-adrenoceptors are located prejunctionally on cholinergic post-ganglionic nerve terminals and β-adrenoceptors postjunctionally on the smooth muscle cells. Activation of the prejunctional α-adrenoceptors results in an inhibition of acetylcholine release, while β-adrenoceptor stimulation produces a direct inhibition of smooth muscle contractility (see Wikberg (1977) and Drew (1978) for further references).

In sympathetically innervated tissues, experiments with a number of α-adrenoceptor agonists and antagonists have shown that pre- and postjunctional α-adrenoceptors differ in their pharmacological characteristics (Starke, 1977). Drew (1978), Wikberg (1978a, b) and Tayo (1979) have suggested that the prejunctional α-adrenoceptors present on cholinergic nerve terminals in the guinea-pig ileum are of a similar type to those found prejunctionally on sympathetic neurones. In the above studies, the authors used the α-adrenoceptor agonists clonidine and oxymetazoline. However, information is lacking on the actions of structurally related compounds.

In the present investigations, 22 imidazolidine (clonidine-like) and 6 imidazoline (oxymetazoline-like) compounds with varying phenyl ring substituents have been compared with noradrenaline for their ability to inhibit responses in transmurally stimulated guinea-pig ileal preparations. The compounds were also monitored for their ability to inhibit acetylcholine-induced contractions in order to determine whether the depressant effects observed might involve activity at postjunctional sites.

The preparations were obtained from reserpine-treated guinea-pigs, since in a number of gastrointestinal preparations it has been shown that activation of sympathetic nerves during transmural stimulation interferes with cholinergic transmission (Paton & Vizi, 1969; Beani, Bianchi & Crema, 1969; Vizi & Knoll, 1971; Knoll & Vizi, 1971). In addition, propranolol was added to the bathing fluid to prevent postjunctional β-adrenoceptor mediated effects. Prejunctional α-adrenoceptor-mediated actions were assessed at low frequencies of stimulation, since in the guinea-pig ileum this action has been shown to be a frequency-dependent phenomenon (Paton & Vizi, 1969; Knoll & Vizi, 1971; Vizi & Knoll, 1971).

All the imidazolidines and imidazolines studied have been shown to possess activity at postjunctional α-adrenoceptor sites in rat vas deferens preparations. Their activity at prejunctional α-adrenoceptor sites

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on sympathetic nerves in guinea-pig atria was variable, both agonistic and antagonistic actions being observed (Malta, Ong, Raper, Tawa & Vaughan, 1980).

Methods

Segments of guinea-pig ileum, obtained from animals pretreated with reserpine (2.5 mg/kg i.p. 24 h), were mounted on a platinum electrode assembly and bathed in Krebs solution (NaCl 120, KCl 7.3, $MgSO_4$ 0.6, dextrose 11.1 $NaHCO_3$ 25, NaH_2PO_4 1.0 and $CaCl_2$ 2.6 mmol/l) maintained at 32°C and aerated with 5% CO_2 in O_2. The tissues were suspended under a tension of 0.5 g and responses were recorded with a Grass FTO3c force displacement transducer coupled to a Grass 79D polygraph. The bathing solution included propranolol (1 μmol/l), mepyramine (1 μmol/l), cimetidine (10 μmol/l) and desipramine (DMI, 0.5 μmol/l) to prevent unwanted actions of the compounds at other sites. In transmurally stimulated preparations, choline (20 μmol/l), was included in the bathing solution to maintain transmitter stores.

Responses to transmural stimulation were elicited at 0.1 Hz with square wave pulses of 2.5 ms duration and a voltage sufficient to produce maximal contractions.

In each experiment, full cumulative concentration-effect curves for the inhibitory actions of the compounds were established at 20 min intervals. Curves to noradrenaline were first obtained and thereafter responses to a test compound were monitored. Responses were expressed as a percentage of the maximal inhibitory response to noradrenaline and pD_2 values and values for intrinsic activity (noradrenaline = 1) were interpolated from the graphical plots. Compounds were classed as inactive if they failed to show signs of inhibitory activity at concentrations up to 0.1 mmol/l. When maximal responses to a compound had been achieved, phentolamine (1 μmol/l) was added to the bathing fluid. A complete reversal of the inhibitory effect was taken as evidence that α-adrenoceptor activation was involved in the response to the compound.

In further experiments, quantitative evaluations of the antagonistic actions of phentolamine and other compounds were obtained. In these experiments, cumulative concentration-effect curves to various agonists were obtained alone and in the presence of increasing concentrations of an antagonist. Antagonism was assessed from shifts in the curves by the method of Arunlakshana & Schild (1959).

An antagonist contact-time of 30 min was used with each antagonist concentration tested.

Since α-adrenoceptor-mediated inhibition of transmitter release at cholinergic neuroeffector junctions is a frequency-dependent phenomenon, selected compounds were tested for inhibitory activity in preparations stimulated transmurally at low (0.125 Hz), medium (8 Hz) and high (32 Hz) frequencies. The duration of stimulation at any particular frequency was adjusted so that the number of pulses delivered (64) was kept constant. After obtaining constant control responses at the three stimulation frequencies, concentrations of the agonists that produced just maximal inhibitory effects at 0.1 Hz stimulation frequency were added to the bathing fluid and frequency-response curves were re-established. Responses were expressed as a percentage of the control contractions obtained at each frequency. Responses to stimulation at 8 and 32 Hz were delivered at 60 and 30 s intervals respectively, since pilot studies showed that contractile responses of the preparations were maintained constant with these stimulation parameters.

In studies where the postjunctional activity of the compounds was assessed, responses to exogenous acetylcholine were obtained. The concentration of acetylcholine used was adjusted so that the submaximal contraction heights obtained were similar to those produced by transmural stimulation of the preparations at 0.1 Hz. The test compounds were added 30 s before an acetylcholine challenge. Pilot experiments showed that with different compounds this agonist contact time was sufficient to allow expression of maximal inhibitory activity. Graphical plots of percentage inhibition versus agonist concentration were made and pD_2 values interpolated.

Compounds were classed as inactive when inhibitory effects did not occur with concentrations of up to 0.1 mmol/l.

The drugs used were cimetidine (Smith, Kline & French); prazosin, desmethylimipramine, tolazoline, phentolamine, naphazoline and xylometazoline (Ciba-Geigy); oxymetazoline (Glaxo-Allenbury); thymoxamine (Warner Lambert); acetylcholine, noradrenaline and yohimbine (Sigma); propranolol (Imperial Chemical Industries); clonidine and the St compounds listed in Table 1 (Boehringer-Ingelheim).

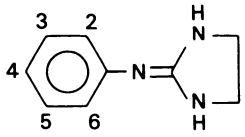
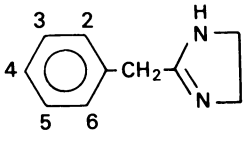
Stock solutions (10 mmol/l) of the compounds were dissolved in 10 mmol/l HCl and suitable dilutions made up in Krebs solution containing 20 mg/l ascorbic acid.

Results

Noradrenaline

Noradrenaline produced a concentration-dependent inhibition of contractions elicited by transmural stimulation (0.1 Hz) of the ileal preparations (mean $pD_2 = 6.94$, s.e.mean = 0.07), maximal concentrations producing a complete inhibition of the re-

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	St 363	(HCl)	2,4-diCl
St 464	(HCl)	2,6-diBr	St 375	(HNO_3)	2-Cl, 4-CH_3
St 1912	(base)	2,6-diF	St 476	(HCl)	2,3-diCl
St 1923	(HCl)	2-Cl, 6-F	St 608	(HCl)	2-Cl, 3-CH_3
St 93	(HCl)	2-Cl, 6-CH_3	Tramazoline	(HCl)	2,3-cyclohexano
St 95	(HCl)	2,6-diCH_3	St 96	(base)	2-Cl
St 1697	(base)	2-CH_3, 6-CH_2CH_3	St 732	(HCl)	2,4, 6-triCl
St 91	(HCl)	2,6-diCH_2CH_3	St 666	(HBr)	2,6-diCl, 4-OH
St 475	(HCl)	2,5-diCl	St 89	(HCl)	2,4, 6-triCH_3
St 585	(HCl)	2-CH_3, 5-Cl	St 1943	(HBr)	3,4-diOH
St 600	(HCl)	2-CH_3, 5-F	St 465	(base)	unsubstituted
Imidazolines					
St 1913	(HCl)	2,6-diCl	Xylometazoline	(HCl)	2,6-diCH_3, 4-$C(CH_3)_3$
Naphazoline	(HCl)	2,3-benzo	Oxymetazoline	(HCl)	2,6-diCH_3, 3-OH, 4-$C(CH_3)_3$
St 71	(HCl)	2,4, 6-triCH_3	Tolazoline	(HCl)	unsubstituted

sponses. In contrast, concentrations of the catecholamine that produced maximal inhibitory effects resulted in only a partial blockade (75 ± 6%) of responses to exogenous acetylcholine (mean $pD_2 = 6.58$, s.e.mean = 0.08).

Phentolamine (1 μmol/l), when added to the bathing fluid in the presence of maximal inhibitory concentrations of noradrenaline, produced a partial reversal (27 to 46%) of the responses to transmural stimulation but was without effect on the inhibitory activity in acetylcholine stimulated preparations.

In further studies, concentration-effect curves to noradrenaline were established in the absence and in the presence of increasing concentrations of yohimbine (Figure 1a) and phentolamine.

The antagonists produced only small shifts to the right of the concentration-effect curves, together with a depression in the maximal responses. Thymoxamine (2 to 10 μmol/l) produced similar effects, the depression in the slopes of the concentration-effect curves and the maximal responses being especially marked with this antagonist. Prazosin (0.1 to 10 μmol/l) and pimozide (0.5 to 1 μmol/l) were without effect on the responses to noradrenaline.

Imidazolidines and imidazolines

The compounds could be divided into four broad groups on the basis of the relative concentrations required to inhibit contractions to transmural stimulation and to exogenous acetylcholine and on the reversibility of the inhibition in the presence of the α-adrenoceptor antagonist, phentolamine (1 μmol/l).

Group 1 The compounds in this group inhibited contractions in transmurally stimulated preparations but had little or no effect on responses elicited by exogenous acetylcholine. The inhibitory activity produced by the compounds was completely reversed by phentolamine.

Table 2 shows the mean intrinsic activities (noradrenaline = 1) and pD_2 values for the inhibitory actions of these compounds in transmurally stimulated preparations, together with their relative activities with respect to noradrenaline (drug EC_{50}: noradrenaline EC_{50}). Figure 2 shows mean concentration-effect curves for some of the compounds within this group. With the exception of clonidine and St 464, none of the compounds at concentrations up to 0.1 mmol/l depressed responses to exogenous acetylcholine. With the former drugs only weak and inconsistent inhibitory responses were observed at concentrations > 1000 times those required to inhibit responses to transmural stimulation (mean clonidine $pD_2 = 3.82$, $n = 2$; mean St 464 $pD_2 = 4.12$, $n = 2$).

None of the imidazolidines or imidazolines pro-

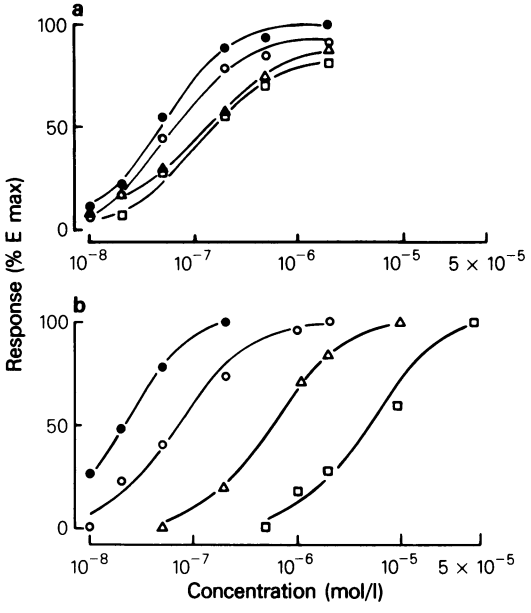


Figure 1 Transmurally stimulated preparation. Concentration-effect curves from an experiment in which inhibitory responses to (a) noradrenaline, and (b) clonidine were assessed alone (●) and in the presence of 0.1 (○), 1.0 (△) and 5.0 (□) μmol/l yohimbine. Results are expressed in terms of the maximal inhibitory effect produced by each agonist under control conditions and individual points show concentrations for a given percentage effect.

duced a complete inhibition of the transmurally elicited contractions. The relative activities of the compounds ranged from approximately five times less to five times more than that of the catecholamine (Figure 2, Table 2).

Further experiments were performed using clonidine, since this drug appeared to be typical of the group as a whole. In contrast to noradrenaline, concentration-effect curves to clonidine were shifted in a parallel fashion by phentolamine and yohimbine (Figure 1b), and the shifts in the curves were not associated with a depression in the maximal responses.

The mean pA_2 values (± s.e.mean) for phentolamine and yohimbine when clonidine was used as the agonist, were 9.04 ± 0.15 ($n = 4$) and 7.51 ± 0.08 ($n = 4$) respectively, and the mean slopes of the relationship between log (dose-ratio -1) and log (antagonist concentration) 1.33 ± 0.30 and 1.36 ± 0.34. Although there was some variability in the shifts obtained in different preparations, it appears that the antagonism is of a competitive type (Arunlakshana & Schild, 1959).

As in the experiments with noradrenaline, prazosin (0.1 to 10 μmol/l) failed to antagonize responses to clonidine, and thymoxamine produced only small shifts of the concentration-effect curves that were associated with a marked depression in the maximal responses.

Group 2 Compounds within this group (Table 2) were similar to the clonidine-like (group 1) drugs, in

Table 2 Effects of group 1 and group 2 compounds in transmurally stimulated guinea-pig ileum preparations

Drug	pD_2	α	AR
Group 1			
Clonidine	7.20 (0.25)	0.45 (0.02)	0.18 (0.05)
St 93	7.30 (0.10)	0.41 (0.03)	0.23 (0.11)
St 1913	6.76 (0.10)	0.29 (0.09)	0.29 (0.09)
Naphazoline	7.57 (0.19)	0.39 (0.04)	0.39 (0.04)
St 464	7.55 (0.09)	0.37 (0.05)	0.60 (0.20)
St 1697	7.16 (0.20)	0.53 (0.06)	1.07 (0.49)
Tramazoline	7.38 (0.14)	0.45 (0.06)	1.47 (0.43)
St 91	6.86 (0.20)	0.76 (0.08)	1.72 (0.44)
St 1923	6.73 (0.03)	0.38 (0.09)	2.32 (0.34)
St 1943	6.77 (0.18)	0.65 (0.08)	4.12 (1.82)
St 95	6.75 (0.39)	0.69 (0.12)	4.75 (2.83)
Group 2			
Oxymetazoline	7.05 (0.08)	0.93 (0.05)	1.16 (0.26)
St 666	5.57 (0.08)	0.51 (0.04)	5.24 (1.3)
St 71	5.51 (0.09)	0.71 (0.16)	37.5 (6.2)
St 476	4.58 (0.32)	> 0.8	411 (202)

Mean pD_2 values, intrinsic activities (α, noradrenaline = 1) and activity ratios (AR, Drug EC_{50}:Noradrenaline EC_{50}) for the inhibitory actions of the compounds are shown. The figures in parentheses are s.e. mean ($n > 4$ with each drug). For St 476, the activity ratio was calculated with respect to the EC_{50} for noradrenaline.

Table 3 pD_2 values for the group 3 compounds on responses of guinea-pig ileum to transmural stimulation and exogenous acetylcholine

Drug	*Transmural stimulation*	*Exogenous acetylcholine*
	pD_2	pD_2
Xylometazoline	5.44 (0.12)	5.37 (0.18)
St 89	5.00 (0.53)	4.56 (0.33)
St 363	4.37 (0.14)	4.34 (0.17)
St 375	4.29 (0.13)	4.49 (0.22)
St 608	5.39 (0.33)	4.48 (0.32)
St 732	4.38 (0.31)	4.18 (0.21)

Mean pD_2 values ($n = 4$ for transmurally stimulated preparations and 3 for exogenous acetylcholine) together with s.e. mean in parentheses.

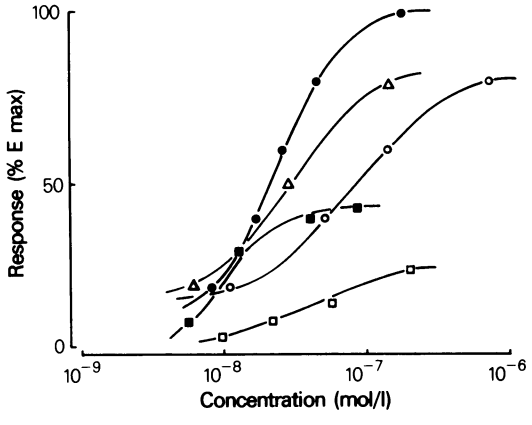


Figure 2 Transmurally stimulated preparations. Mean concentration effect curves ($n > 4$) to noradrenaline (●), St 91 (△), St 95 (○), clonidine (■) and St 1913 (□). Results are expressed in terms of the maximal inhibitory response to noradrenaline (E max) and individual points show mean concentrations for a given percentage effect.

that they inhibited responses to transmural stimulation while having little effect on responses to exogenous acetylcholine. St 71 and St 476 had no inhibitory effects on acetylcholine-induced contractions when used at concentrations up to 0.1 mmol/l. Oxymetazoline and St 666 displayed only weak anti-acetylcholine activity. Although their intrinsic activities approached that of noradrenaline (= 1), their pD_2 values for inhibition of acetylcholine-induced responses were some two orders of magnitude less than those required for inhibition of responses to transmural stimulation (St 666, $\alpha = 0.83$, $pD_2 = 3.95$, $n = 2$; oxymetazoline, $\alpha = 0.83$, $pD_2 = 4.85$, $n = 2$). The inhibitory effects of these two compounds were unaffected by phentolamine (1 μmol/l).

The main difference between the Group 2 and Group 1 compounds resided in their interaction with phentolamine in transmurally stimulated preparations. While phentolamine (1 μmol/l) produced a complete reversal of the maximal inhibitory responses to the group 1 compounds, it failed to reverse the inhibitory effects produced by St 71 and St 475, and only partially reversed the inhibitory actions of St 666 (75 ± 5%) and oxymetazoline (53 ± 6%).

In further experiments with the latter compounds, it was shown that the antagonism produced by phentolamine was not competitive. The concentration-effect curves displayed characteristics similar to those previously described for the interaction of noradrenaline and phentolamine in transmurally stimulated preparations.

Group 3 The main characteristic of this group (Table 3) was that the compounds produced inhibitory effects against contractions elicited by both transmural stimulation and exogenous acetylcholine. Furthermore, similar pD_2 values for inhibitory effects (Table 3) were obtained in both types of preparation and contractile responses were completely abolished. In this respect, the group 3 compounds are somewhat similar to noradrenaline. However, unlike noradrenaline their inhibitory effects in transmurally stimulated preparations were completely unaffected by phentolamine (1.0 μmol/l).

Group 4 At concentrations up to 0.1 mmol/l, compounds within this group (St 96, St 465, St 585, St 600, St 1912 and tolazoline) were without effect on responses to both transmural stimulation and exogenous acetylcholine.

Within this group of compounds, those without phenyl ring substituents (St 465 and tolazoline) possessed affinity for α-adrenoceptors, in that they competitively antagonized responses to clonidine in transmurally stimulated preparations (St 465, $pA_2 = 6.89 \pm 0.09$, slope = 1.25 ± 0.20, $n = 3$; to-

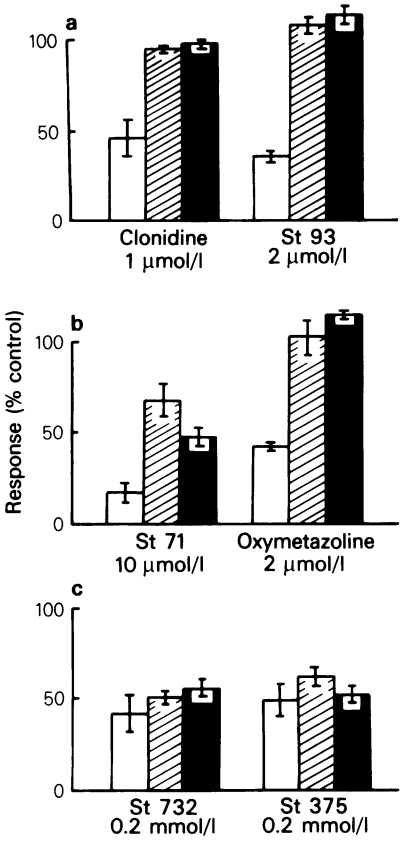


Figure 3 Transmurally elicited responses in guinea-pig ileum preparations stimulated at 0.125 (open columns), 8 (hatched columns) and 32 Hz (solid columns). Histograms show mean responses ($n = 4$) expressed as a percentage of control in the presence of the indicated concentrations of the compounds; vertical lines show s.e. mean.

lazoline, $pA_2 = 6.23 \pm 0.27$, slope $= 0.88 \pm 0.05$, $n = 4$).

Effects obtained at varying frequencies of stimulation

In an attempt to delineate further the mechanisms by which the compounds might produce their inhibitory effects, their ability to affect responses elicited at differing frequencies of transmural stimulation were tested.

Contractions elicited at 8 and 32 Hz were $156 \pm 11\%$ and $87 \pm 3\%$ respectively greater than those obtained at the lowest frequency of stimulation used in these experiments (0.125 Hz).

In addition to noradrenaline, the compounds studied were clonidine and St 93 (group 1); St 71, St 476, St 666 and oxymetazoline (group 2) and xylometazoline, St 89, St 375, St 608 and St 732 (group 3).

In concentrations that produced maximal inhibitory responses at 0.1 Hz, noradrenaline reduced responses elicited at all three frequencies of stimulation, while the group 1 compounds (clonidine and St 93) produced a specific inhibition of low frequency responses (Figure 3a).

Of the group 2 compounds, St 71 (Figure 3b), St 476 and St 666 reduced responses to all three stimulation frequencies, while oxymetazoline specifically inhibited responses to low frequency stimulation (Figure 3b).

All the group 3 compounds tested, produced a depression of the responses elicited at all three stimulation frequencies. Figure 3(c) shows the effects produced by two of these compounds, St 732 and St 375.

Discussion

Wikberg (1977) has previously shown that in guinea-pig ileal preparations, noradrenaline produces an inhibitory effect against contractions induced by acetylcholine that does not involve adrenoceptor activation. This was confirmed in the present studies and extended to many of the imidazolidines and imidazolines used, which, on the basis of previous studies (Malta *et al.*, 1980) have been shown to possess postjunctional α-adrenoceptor stimulant properties.

In the present studies, pD_2 values of a similar order were obtained for noradrenaline-induced inhibition of transmural and acetylcholine-induced responses. Although the α-adrenoceptor antagonists, phentolamine and yohimbine inhibited the noradrenaline-induced effects in transmurally stimulated preparations, the antagonism was incomplete and did not fulfil the requirements for a competitive interaction. The results obtained with phentolamine are in accord with those of Wikberg (1978a), however Tayo (1979) has suggested that a competitive interaction exists between yohimbine and noradrenaline in transmurally stimulated preparations. The reasons for these discrepant results are unknown. The non-competitive nature of the interactions between noradrenaline and the α-adrenoceptor antagonists, phentolamine and yohimbine, probably results from the mixed prejunctional (α-adrenoceptor mediated) and postjunctional (non α-adrenoceptor mediated) effects of the agonist, since both effects occur over a similar concentration range.

Of the 28 imidazolidines and imidazolines studied, 21 produced an inhibition of the responses to transmural stimulation; however, the mechanisms underlying this effect differed.

Clonidine and the remaining compounds in group 1 appear to have selective actions at prejunctional α-adrenoceptors and presumably inhibit contractily through depression in acetylcholine release.

This view is supported by the fact that effective inhibitory concentrations of the compounds have no action against responses to exogenous acetylcholine and that the effects are only observed at low frequencies of stimulation and are reversed by phentolamine. Furthermore, phentolamine and the selective prejunctional α-adrenoceptor antagonist, yohimbine (see Wikberg (1979) for references), display competitive interactions. The absence of effects with prazosin, which displays selective postjunctional α-adrenoceptor antagonistic effects (Wikberg, 1979) also supports the view that prejunctional α-adrenoceptors are involved in the observed inhibitory actions. Overall, these results support the proposals made by Drew (1978) and Wikberg (1979), that prejunctional α-adrenoceptors on cholinergic and noradrenergic nerve terminals have similar characteristics.

In view of the suggestion that thymoxamine has selective postjunctional α-adrenoceptor antagonistic actions (Wikberg, 1979), its ability to inhibit responses to clonidine is unexpected. However, the antagonism observed was not of a competitive type and may be related to other pharmacological actions of the compound (Drew, 1978).

Oxymetazoline and the compounds in group 2 resembled clonidine and the group 1 compounds in terms of their selective reduction in responses to transmural stimulation rather than to acetylcholine. However, the frequency-dependence in their activity was less marked and phentolamine either failed or produced only a partial reversal of their inhibitory effects. These results raise the possibility that their inhibitory activity involves a reduction in acetylcholine release through a neuronal depressant action in which α-adrenoceptor-mediated actions play either no role (St 71, St 476) or only a minor role (oxymetazoline, St 666).

Although xylometazoline and the other compounds in group 3 depress responses to transmural stimulation, their effects are not antagonized by phentolamine and are not frequency-dependent. In view of the fact that responses to exogenous acetylcholine and transmural stimulation are inhibited by similar concentrations of the compounds, their actions could be accounted for by a postjunctional smooth muscle depressant action which does not involve the activation of α-adrenoceptors. The concentrations of these compounds required to inhibit transmurally elicited responses (range of pD_2 values 4.29 to 5.44) are considerably higher than those of the group 1 compounds required to produce selective prejunctional α-adrenoceptor-mediated effects (pD_2 range 6.73 to 7.57). The possibility that a weak α-adrenoceptor-mediated inhibition of transmitter release may play a minor role in their actions cannot be eliminated on the basis of the present experimental results.

In previous studies with these imidazolines and imidazolidines, structure-activity relationships indicate that activity at postjunctional α-adrenoceptors demands a molecular orientation in which the phenyl and the imidazoline or imidazolidine rings are in different planes, maximal stimulant activity being obtained with compounds having substitutions in the 2 and 6 positions of the phenyl ring (Malta *et al.*, 1980). These features are also apparent in the present study when considering the selective prejunctional α-adrenoceptor-mediated actions of the compounds on cholinergic nerves. However, 2,6-phenyl substitution is not an absolute prerequisite, since selective prejunctional α-adrenoceptor actions are also seen with the compounds possessing a cyclized moeity in the 2,3 position, i.e. tramazoline (2,3 cyclohexano) and naphazoline (2,3-benzo).

Although the mechanism(s) by which non-adrenoceptor mediated pre- and postjunctional depressant actions are produced is unknown, it would appear that within groups 2 and 3, tri-substitution of the phenyl ring or di-substitution in the 2,3 or 2,4 positions is a characteristic for this type of activity.

Absence of both depressant and α-adrenoceptor-mediated stimulant activity is obtained with the mono substituted compound, St 96, and the 2,5-disubstituted compounds St 495, St 585 and St 600. Prejunctional α-adrenoceptor antagonistic actions occur with the unsubstituted compounds St 465 and tolazoline. The absence of α-adrenoceptor-mediated activity with the 2,6-difluoro substituted compound St 1912 is somewhat unexpected, since it has previously been shown to possess postjunctional α-adrenoceptor stimulant actions (Malta *et al.*, 1980). However, at prejunctional α-adrenoceptors on sympathetic nerves in guinea-pig atria it is without obvious effects (Malta *et al.*, 1980); this absence of prejunctional α-adrenoceptor activity has been confirmed in the present studies.

In conclusion, the results of the present studies confirm and extend those of our own and other authors who have suggested that prejunctional α-adrenoceptors on cholinergic and adrenergic nerves have similar characteristics. The importance of 2,6-phenyl substitution for such activity in imidazolines and imidazolidines has also been highlighted.

The fact that drugs within these classes can inhibit responses to transmural stimulation of the guinea-pig ileum by differing mechanisms emphasizes the need for caution in assuming that prejunctional α-adrenoceptor stimulation is involved. Corroborative evidence obtained with α-adrenoceptor antagonists, investigations into frequency-response relationships, assessment of effects in preparations stimulated with exogenous acetylcholine and transmitter overflow studies are required before the actions of a compound can be assigned to this mechanism with any confidence.

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