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RECEPTOR INTERACTIONS OF IMIDAZOLINES: α-ADRENOCEPTORS OF RAT AND RABBIT AORTAE DIFFERENTIATED BY RELATIVE POTENCIES, AFFINITIES AND EFFICACIES OF IMIDAZOLINE AGONISTS

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1 Noradrenaline and a series of imidazolines were used to characterize and differentiate the postsynaptic α-adrenoceptors of rat and rabbit aortae.

2 Dose-response curves in each tissue revealed marked differences in the profile of agonist activity among the compounds. Based on the ED_{50} values for each compound, a rank order of potency of oxymetazoline > noradrenaline > tramazoline > tetrahydrozoline > clonidine was obtained in rabbit aorta and an order of noradrenaline > clonidine > tramazoline > oxymetazoline was obtained in rat aorta. Tetrahydrozoline had no agonist activity in rat aorta.

3 Dissociation constants were determined for each agonist in rat and rabbit aortae. Again, differences between the tissues were observed to the extent that the rank order of affinities for the imidazolines were exactly opposite for the two tissues. In rabbit aorta the order was, oxymetazoline > tramazoline > tetrahydrozoline > clonidine, whereas in rat aorta it was, clonidine > tetrahydrozoline > tramazoline > oxymetazoline. The extremes in tissue selectivity were observed with clonidine, which had approximately 125 fold higher affinity in rat aorta, and oxymetazoline, which had approximately 4 times higher affinity in rabbit aorta.

4 The absolute values of relative efficacies of the imidazolines studied, and their rank order, also differed between the two tissues. The relative efficacies of oxymetazoline and tramazoline were more than 15 fold greater in rabbit aorta than in rat aorta. Furthermore, tetrahydrozoline had a greater relative efficacy than clonidine in rabbit aorta while the converse was true in rat aorta.

5 Differences in the rank order of potency, affinity and relative efficacy of noradrenaline and a series of imidazolines in rat and rabbit aortae indicate that the postsynaptic α-adrenoceptors in these tissues are different. While the postsynaptic α-adrenoceptor of rabbit aorta is clearly of the α_1-subtype, the exact nature of the postsynaptic α-receptor of rat aorta is not clear. The unique α-receptor of rat aorta has properties of both α_1- and α_2-adrenoceptors.

Introduction

We have recently shown that the postsynaptic α-adrenoceptor of rat aorta differs from the postsynaptic α-receptor in a variety of other tissues from the rat (Ruffolo, Yaden & Waddell, 1980b; Ruffolo, Waddell & Yaden, 1981a). The α-receptor in rat aorta also differs from the receptor in aorta of other mammalian species (Ruffolo, Waddell & Yaden, 1982). In these studies, we used the α_2 selective agonist, clonidine, and antagonist, yohimbine, to differentiate α-receptors in rat aorta from the postsynaptic α-receptors located in other tissues. The uniqueness of the α-adrenoceptor in rat aorta has since been confirmed by other laboratories (Randriantsoa, Keitz & Stoclet, 1981; Downing, Wilson & Wilson, 1981).

The α-adrenoceptor of rat aorta has many properties of α_2-adrenoceptors (Ruffolo *et al.*, 1980b; 1981a), and in many instances its response is highly predictive of α_2-adrenoceptor effects (Ruffolo, Yaden & Waddell, 1981b; Ruffolo *et al.*, 1982). Several of the procedures developed to subclassify α-adrenoceptors (Wikberg, 1978) also suggested the α_2 nature of the receptor in rat aorta (Ruffolo *et al.*, 1981a; 1982). These observations led us to postulate that the α-receptor of rat aorta was of the α_2-subtype (Ruffolo *et al.*, 1981a). However, the high potency in this tissue of prazosin (Doggrell & Paton, 1978; Furchgott, 1980; Downing *et al.*, 1981; Randriantsoa *et al.*, 1981) and phenylephrine (Ruffolo, Rosing & Waddell, 1979c; Ruffolo, Waddell & Yaden, 1980a; Ruffolo *et al.*, 1980b), both selective for α_1-receptors, casts doubt upon our original subclassification. We have therefore recently proposed that the α-adrenoceptors of rat aorta possess properties of both α_1- and α_2-receptors, and particularly the latter

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(Ruffolo *et al.*, 1982), and is therefore different from α_1- and α_2-adrenoceptors of other tissues (Drew, 1978; Starke & Docherty, 1980). Although the similarity of the α-receptor in rat aorta to α_2 far exceeds that for α_1 (Ruffolo *et al.*, 1982), it appears that the receptor in this tissue cannot be forced into the α_1/α_2 subclassification.

The rat and rabbit aortae are both tissues commonly used to evaluate postsynaptic α-adrenergic effects. It is clear that the α-receptor of rabbit aorta is α_1 (Docherty, Constantine & Starke, 1981; Ruffolo *et al.*, 1982). Results to date indicate that it is important not to equate these two tissues. Our previous study differentiating α-receptors in rat and rabbit aorta (Ruffolo *et al.*, 1982) involved only two compounds (i.e., clonidine and yohimbine) which is too few to classify receptors. In the present study we are attempting to distinguish further between the α-receptor in rat and rabbit aortae (and at the same time characterize these receptors) by using a series of agonists of the imidazoline class. By studying a series of agonists, we are attempting to differentiate α-receptors in rat and rabbit aortae using several methods of receptor classification (Furchgott, 1972): (1) relative potency of a series of agonists, (2) comparison of dissociation constants, and (3) comparison of relative efficacies of a series of agonists. The compounds used were chosen on the bases of previous studies which suggested they would be particularly useful in contrasting the α-receptors in these two tissues. Noradrenaline was included as a standard reference agonist.

Methods

Male albino rats (Harlan Wistar, 250 to 425 g) and male albino rabbits (Langshaw, 2 to 2.5 kg) were killed by a sharp blow to the head. Segments of thoracic aorta were removed and dissected free of fat and connective tissue in physiological salt solution (PSS, pH 7.40) at room temperature. Helically cut strips, approximately 2 mm wide and 30 mm long, were prepared as described by Furchgott & Bhadrakom (1953). Aortic strips were suspended in 10 ml organ baths containing PSS maintained at 37.5°C and aerated with a 5% CO_2:95% O_2 mixture. The composition of PSS was (mM): NaCl 118, KCl 4.7, $MgCl_2$ 0.54, $CaCl_2$ 2.5, NaH_2PO_4 1.0, $NaHCO_3$ 25 and glucose 11 dissolved in demineralized water. In all cases, PSS contained cocaine (10^{-5} M), propranolol (10^{-6} M), and ethylenediamine tetraacetic acid (EDTA, 3×10^{-5} M) to inhibit neuronal uptake, block β-adrenoceptors, and prevent the spontaneous oxidation of catecholamines, respectively. The tissues were attached to Grass FT-03 isometric transducers which were connected to a Grass Model 7 Polygraph recorder, and allowed to equilibrate under appropriate resting tensions (rat 2 g; rabbit 5 g) for at least 2 h before drug addition. In all experiments, at least one aortic strip run in parallel with the experimental strips, but receiving no antagonists, was used to correct for time-dependent changes in agonist sensitivity (Furchgott, 1972).

Dose-response curves

Dose-response curves to noradrenaline and the imidazolines were constructed by the method of stepwise cumulative addition of agonist (Van Rossum, 1963). The concentration of agonist in the muscle chamber was increased approximately 3 fold at each step, with each addition being made only after the response to the previous addition had attained a maximum level and remained steady. After completion of a dose-response curve, drugs were washed from the preparation at regular intervals by the overflow method. Consecutive dose-response curves on a given tissue were separated by at least 2 h to insure maximum washout of agonist and to minimize the possibility of receptor desensitization. All responses to the imidazolines were expressed as a percentage of the noradrenaline maximum which was obtained from a noradrenaline dose-response curve prior to construction of the dose-response curves to the imidazolines.

Determination of dissociation constants

Dissociation constants of partial agonists (K_P) were obtained by the technique of Waud (1969). In a given tissue, a dose-response curve was first constructed for noradrenaline and then for the partial agonist. The relationship between the dose-response curves of a strong agonist (noradrenaline) and a partial agonist is described mathematically by the following equation (Waud, 1969):

$$\frac{1}{[A]} = \frac{e_A}{K_A e_P} + \frac{K_P e_A}{K_A e_P [P]} \qquad (1)$$

where [A] and [P] are, respectively, equieffective concentrations of strong agonist and partial agonist, K_A and K_P are dissociation constants for the strong agonist and partial agonist, respectively, and e_A and e_P represent efficacies of the strong and partial agonists, respectively. The K_P is readily obtained from the linear plot of 1/[A] against 1/[P] by the equation (Waud, 1969):

$$K_P = \text{slope/intercept} \qquad (2)$$

The technique of Waud (1969) may be used only when $e_A \gg e_P$ or when $ED_{50} \ll K_A$. The data in Figure 2 and Table 3 indicate that this criterion is satisfied in the present investigation.

The technique of Waud (1969) described above is not applicable for the full agonist, noradrenaline. Nor is it applicable for oxymetazoline in rabbit aorta since the dose-response curve for this agonist lies to the left of the noradrenaline curve (see Figure 1a). For these compounds, the procedure of Furchgott & Bursztyn (1967) was used. According to Furchgott & Bursztyn (1967), the following relationship exists between the dose-response curves of an agonist before and after partial receptor inactivation with an irreversible antagonist such as dibenamine (N,N-dibenzyl-β-chloroethylamine, 10^{-6} M for 7 min):

$$\frac{1}{[A]} = \frac{1-q}{qK_A} + \frac{1}{q[A']} \quad (3)$$

where [A] and [A'] are corresponding equieffective concentrations of agonist before and after partial receptor inactivation, respectively, and q is the fraction of active receptors remaining (receptors not alkylated) after partial irreversible blockade. The dissociation constant (K_A) is obtained from the linear plot of 1/[A] vs 1/[A'] by the following equation:

$$K_A = (\text{slope} - 1)/\text{intercept} \quad (4)$$

For those compounds that were extremely weak partial agonists (i.e., tetrahydrozoline in rat aorta) for which the techniques described above were inadequate, a different procedure was used, also described by Furchgott & Bursztyn (1967), in which the partial agonist serves as a competitive antagonist of noradrenaline (full agonist) after partial inactivation of the α-receptor pool with dibenamine (10^{-7} M, 10–20 min). This treatment reduces the response of the partial agonist to a greater extent than the full agonist (Stephenson, 1956; Furchgott, 1966; Triggle & Triggle, 1976), thereby creating the situation where the partial agonist response is insignificant with respect to the full agonist response (Furchgott & Bursztyn, 1967). Under these conditions, the partial agonist may be used as a simple competitive antagonist whose dissociation constant (K_B) is determined by the technique of Arunlakshana & Schild (1959) using noradrenaline (strong agonist) as the agonist. The incubation period of the partial agonist (antagonist) was 60 min in these experiments. For a complete illustration of this technique, the reader may refer to an earlier publication (Ruffolo *et al.*, 1980b).

We have previously shown that the different procedures described above to obtain dissociation constants yield equivalent results in our hands (Ruffolo, Dillard, Waddell & Yaden, 1979a; 1979b; Ruffolo *et al.*, 1979c; 1980a) in accord with receptor theory (Furchgott & Bursztyn, 1967). The various graphical procedures described above for determining dissociation constants of full agonists, partial agonists and competitive antagonists in isolated aortae were performed with the aid of a computer and digital plotter as previously reported in detail (Zaborowsky, McMahon, Griffin, Norris & Ruffolo, 1980).

Determination of relative efficacies of agonists

Efficacies of the imidazolines relative to noradrenaline were determined from the respective equilibrium dissociation constants according to previously published procedures (Furchgott & Bursztyn, 1967; Besse & Furchgott, 1976). Fractional receptor occupancy for each agonist at each concentration [A] was calculated from the following relation (Besse & Furchgott, 1976).

$$\frac{[RA]}{[R_T]} = \frac{[A]}{K_D + [A]} \quad (5)$$

where [RA] is the concentration of receptor-agonist complex, [R_T] is the total receptor concentration and K_D is the dissociation constant of the agonist (determined by one of the previously described procedures). The control response for each agonist is then replotted as a function of the logarithm of fractional receptor occupation ([RA]/[R_T]) and the appropriate curves are constructed. The antilog of the distance along the abscissae between the noradrenaline curve and an imidazoline curve represents the ratio of the efficacy of the imidazoline (e_r) relative to noradrenaline (Besse & Furchgott, 1976).

As an internal check, the e_r for each imidazoline was also calculated after determination of the agonist dissociation constant for the partial agonist as described by Waud (1969) (see equation 4) using the K_A for noradrenaline (estimated by the procedure of Furchgott & Bursztyn, 1967) and the intercept of the plot of 1/[A] vs 1/[P] by the following relationship:

$$e_r = \frac{1}{\text{intercept } K_A} \quad (6)$$

This relationship is a rearrangement of equation 1 made by Ruffolo *et al.* (1979c) and has been shown to yield identical results (Ruffolo *et al.*, 1979a,b) to the method of Furchgott & Bursztyn (1967). For further details on the various methods used to determine agonist and antagonist dissociation constants and the relative efficacies of agonists, the reader may refer to an earlier publication (Ruffolo, *et al.*, 1979c).

Statistical evaluation

The results are expressed as the mean ± s.e.mean. Statistical differences between two means ($P < 0.05$) were determined by Student's t test for unpaired observations or by testing for over lap of 95% confidence limits (Sokal & Rohlf, 1969). All straight lines were drawn by linear regression (Woolf, 1968) and tested, wherever possible, for deviations from linearity by analysis of variance in regression (Sokal & Rohlf, 1969).

Drugs

All drugs were prepared daily in demineralized water or saline. The following drugs were used: (−)-noradrenaline HCl (Sterling-Winthrop), clonidine HCl and tramazoline HCl (Boehringer-Ingelheim), oxymetazoline HCl (Schering Corp.), tetrahydrozoline HCl (Pfizer Inc.).

Results

Dose-response curves

Dose-response curves for noradrenaline and a series of imidazolines in rabbit and rat aortae are presented in Figure 1a and b, respectively. In both tissues, the imidazolines are partial agonists relative to noradrenaline. It is clear from the figure that the profile of activity for the series of agonists differs between rat and rabbit aortae. Important characteristics of the dose-response curves for this series of compounds in rat and rabbit aortae are listed in Table 1.

One method of receptor subclassification is the comparison of relative potencies of a series of agonists (Ahlquist, 1966; Furchgott, 1972). Relative potencies based on ED_{50} values for the series of agonists (Table 1) differ markedly between the two tissues, as do maximum contractile effects (E_{max}), suggesting that the postsynaptic α-adrenoceptors in these tissues differ. The $-\log ED_{50}$ values of 7.97 and 6.15 for oxymetazoline and tetrahydrozoline, respectively, in rabbit aorta agree well with the values of 8.43 and 6.56 reported by Sanders, Miller & Patil (1975) in the same tissue. One major difference we have observed between the rat and rabbit aortae is that in rabbit aorta, oxymetazoline is approximately 7 times more potent than noradrenaline whereas in rat aorta, oxymetazoline is approximately 13 fold less potent than noradrenaline (Table 1). This difference in potency of oxymetazoline relative to norad-

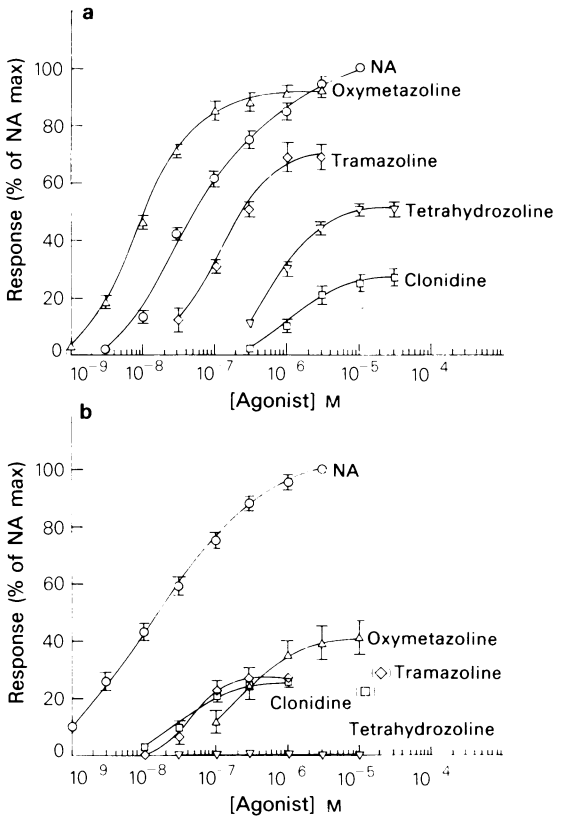

Figure 1 Dose-response curves to noradrenaline (NA) and a series of imidazolines in rabbit (a) and rat (b) aortae. All responses are expressed as a percentage of the maximum noradrenaline response. In (a) $n = 4-7$; in (b) $n = 5-8$.

Table 1 Important parameters of dose-response curves of noradrenaline and a series of imidazolines in rabbit and rat aortae

	Rabbit Aorta				*Rat aorta*			
Compound	n	E_{max}	$-\log ED_{50}$	*Relative potency*	n	E_{max}	$-\log ED_{50}$	*Relative potency*
Noradrenaline	7	1.00	7.15 ± 0.05	1.00	8	1.00	7.77 ± 0.07	1.00
Oxymetazoline	5	0.93 ± 0.03	7.97 ± 0.02	6.61	8	0.41 ± 0.06	6.66 ± 0.09	0.08
Tramazoline	4	0.69 ± 0.05	6.93 ± 0.03	0.60	5	0.26 ± 0.04	7.39 ± 0.05	0.42
Tetrahydrozoline	5	0.51 ± 0.03	6.15 ± 0.03	0.10	5	0	n.a.	n.a.
Clonidine	5	0.27 ± 0.03	5.26 ± 0.07	0.04	7	0.26 ± 0.02	7.62 ± 0.02	0.71

n = number of observations; E_{max} = maximum contractile response relative to noradrenaline; n.a. = not applicable since no contractile response was observed.

renaline is approximately 80 fold between these two tissues.

Furchgott (1972) has shown that problems may arise when comparing ED_{50} values of agonists with differing efficacies. The E_{max} values range from 0.27 to 1.00 in rabbit aorta and from 0 to 1.00 in rat aorta, suggesting that the efficacies vary considerably among these compounds in both tissues, making the comparison of relative potencies based on ED_{50} values tenuous. In addition, differences in E_{max} values between tissues could result from differences in receptor number or differences in the efficiency of coupling between occupancy and response (Stephenson, 1956), and do not necessarily reflect differences in receptor type. While receptor number and coupling will account for absolute differences in E_{max} values between tissues, they cannot account for the differences that we observed in the rank order of E_{max} values within this series of compounds, as noted above. Furthermore, tetrahydrozoline has an E_{max} value greater than clonidine in rabbit aorta, but has an E_{max} of zero in rat aorta. This reversal in E_{max} values cannot be explained by a difference in receptor number or coupling, and as such, would support the view that the α-receptors in these tissues differ.

Dissociation constants

A more reliable method for differentiating receptors is comparison of dissociation constants (Schild, 1947; Furchgott, 1972). This method avoids the problems associated with comparing ED_{50} values among compounds with differing efficacies. It is apparent from Table 2 that the affinities of the imidazolines for α-receptors in rat and rabbit aortae are vastly different in absolute terms, and the rank order of affinities of the imidazolines is exactly opposite in the two tissues (i.e., oxymetazoline > tramazoline > tetrahydrozoline > clonidine in rabbit aorta and clonidine > tetrahydrozoline > tramazoline > oxymetazoline in rat aorta). Clonidine and tetrahydrozoline have approximately 100 times higher affinity in rat aorta than rabbit aorta, and tramazoline shows a 16 fold selectivity for rat aorta over rabbit aorta. Conversely oxymetazoline shows a 4 fold selectivity for rabbit aorta over rat aorta.

Relative efficacies

In addition to affinity, another important parameter of agonist activity is efficacy (Stephenson, 1956; Furchgott, 1966) which relates to the ability of an agonist to stimulate or activate the receptor subsequent to, and independent of (Ariens, 1954), binding to the receptor. While determination of absolute efficacies or intrinsic efficacies is not possible, it is possible to determine the efficacy of one agonist relative to another standard reference agonist (Stephenson, 1956; Besse & Furchgott, 1976). Unlike estimates of E_{max} values which are affected by receptor number or coupling, both of which may differ among tissues, relative efficacies are not affected by these factors and as such, may be used to characterize and differentiate receptor types in different tissues. Noradrenaline is often used as a reference agonist (Besse & Furchgott, 1976). In Figure 2 are plotted the responses of noradrenaline and the imidazolines in rabbit aorta (a), and rat aorta (b) as a function of the logarithm of the percentage of α-adrenoceptors occupied, the latter being calculated from the equilibrium dissociation constants presented in Table 2. Relative efficacies were determined by the technique of Furchgott & Bursztyn (1967) by dividing the percentage of receptors occupied by noradrenaline when producing a response equivalent to 20% of the maximum by the percentage of receptors occupied by the imidazolines when

Table 2 Dissociation constants of noradrenaline and a series of imidazolines in rabbit and rat aortae

	Rabbit aorta			*Rat aorta*		
Compound	n	$-\log K_D$	*Relative affinity*	n	$-\log K_D$	*Relative affinity*
Noradrenaline	5	5.93 ± 0.09	1.00	5	6.58 ± 0.17	1.00
Oxymetazoline	6	6.80 ± 0.21	7.41	5	6.17 ± 0.05	0.39
Tramazoline	4	6.05 ± 0.08	1.32	5	7.25 ± 0.12	4.67
Tetrahydrozoline	5	5.39 ± 0.13	0.29	15	7.33 ± 0.09	5.62
Clonidine	5	5.31 ± 0.04	0.24	7	7.41 ± 0.09	6.76

n = number of individual observations.

Dissociation constants determined by the method of Waud (1969).for partial agonists [oxymetazoline (rat aorta), tramazoline, tetrahydrozoline (rabbit aorta) and clonidine], the technique of Furchgott & Bursztyn (1967) for full agonists such as noradrenaline, and for oxymetazoline in rabbit aorta (see Methods), and by the technique of Furchgott & Bursztyn (1967) for partial agonists for tetrahydrozoline in rat aorta.

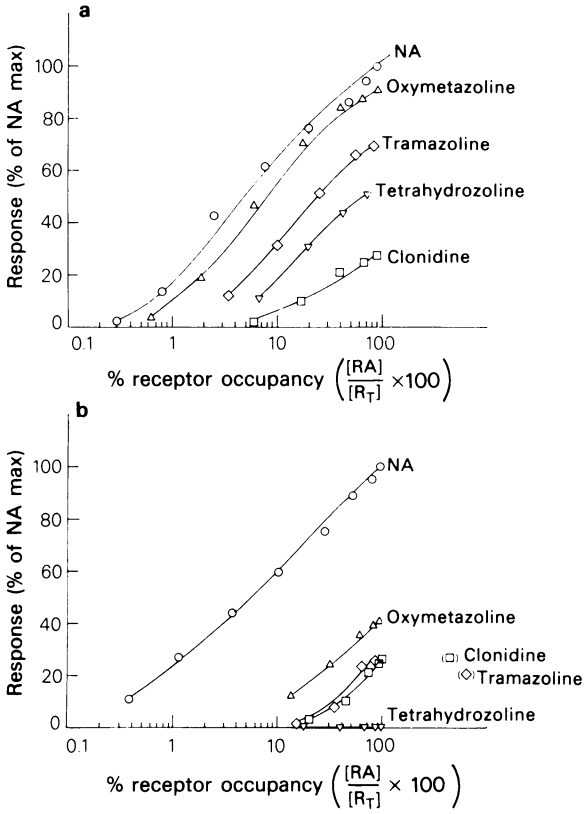

Figure 2 Response to noradrenaline (NA) and a series of imidazolines (from Figure 1) plotted as a function of the logarithm of % receptor occupancy in rabbit (a) and rat (b) aortae. Percentage receptor occupancy was calculated as described in the Methods section. All responses are expressed as a percentage of the maximum noradrenaline response.

producing the same response. As an additional check, relative efficacies of the imidazolines were also determined by another technique we have developed for partial agonists (Ruffolo *et al.*, 1979c) which involves rearrangement of the theoretical considerations presented by Waud (1969). We have previously shown that these two procedures yield similar results (Ruffolo *et al.*, 1979a, b) as is also the case in the present study. The relative efficacies of the compounds studied are presented in Table 3.

The relative efficacies of the imidazolines differ markedly both in absolute terms and in rank order between the two tissues. The largest discrepancies were observed with oxymetazoline and tramazoline whose relative efficacies are more than one order of magnitude greater in rabbit aorta than rat aorta. Furthermore, tetrahydrozoline has a greater relative efficacy than clonidine in rabbit aorta, while the converse is true in rat aorta. In fact, tetrahydrozoline did not produce a measurable response in this tissue, and only a relative efficacy of <0.004 could be estimated, and this is consistent with an earlier study (Ruffolo *et al.*, 1979c). The relative lack of response of tetrahydrozoline in rat aorta has been a consistent observation in our laboratory (Ruffolo *et al.*, 1979c; Ruffolo, Yaden, Waddell & Dillard, 1980c).

Discussion

We have previously concluded that the postsynaptic α-adrenoceptor of rat aorta is different from postsynaptic α-receptors located in other smooth muscles of the rat (Ruffolo *et al.*, 1980b; 1981a) or in aortae from other mammalian species (Ruffolo *et al.*, 1982). The α-adrenoceptor of rat aorta is unique in that it has properties of both α_1- and α_2-adrenoceptors (and particularly the latter; Ruffolo *et al.*, 1980b; 1981a; 1982), and may represent a third subtype of α-

Table 3 Relative efficacies of noradrenaline and a series of imidazolines determined by two techniques in rabbit and rat aortae

	Relative efficacies (e_r)			
	Rabbit aorta		*Rat aorta*	
Compound	Furchgott & Bursztyn (1967)	Waud (1969)	Furchgott & Bursztyn (1967)	Waud (1969)
Noradrenaline	1.000	1.000	1.000	1.000
Oxymetazoline	0.575	n.a.[1]	0.033	0.034 ± 0.004
Tramazoline	0.211	0.305 ± 0.072	0.013	0.022 ± 0.010
Tetrahydrozoline	0.103	0.127 ± 0.012	<0.004	n.a.[2]
Clonidine	0.027	0.028 ± 0.007	0.011	0.021 ± 0.003

n.a.[1] = not applicable since the dose-response curve for oxymetazoline lies to the left of the noradrenaline curve in this tissue.
n.a.[2] = not applicable since the compound failed to produce a response.

receptor or simply a subtype of postjunctional α-receptors (see McGrath, 1982). This α-adrenoceptor of rat aorta may be similar to the unusual α-adrenoceptor observed in dog saphenous vein (Sullivan & Drew, 1980; De Mey & Vanhoutte, 1981). The existence of α-receptors differing from the classical α_1 and α_2 subtypes has also been observed in human vascular tissue (Stevens & Moulds, 1981). Barker, Harper & Hughes (1977) have provided additional evidence for the existence of three types of postsynaptic α-adrenoceptors. Sheys & Green (1972) and Harper, Hughes & Noormohamed (1978) were able to distinguish at least two types of postsynaptic α-receptors, none of which resembles α_2, and this would also suggest the possibility of a third subtype of α-adrenoceptor.

Since agonists may be useful in characterizing receptors (Ahlquist, 1966; Furchgott, 1972), we decided to study a series of α-agonists in rat and rabbit aortae in an attempt to confirm our previous conclusions that the postsynaptic α-adrenoceptors in these tissues are different and should therefore not be equated.

Relative potencies of agonists is an effective method for classifying receptors (Ahlquist, 1966; Furchgott, 1972). In the present investigation, the relative potencies (based on ED_{50} values obtained from dose-response curves) of a series of imidazolines differed markedly between rat and rabbit aortae suggesting that the receptors are different. However, the dose-response curves indicated that the compounds possessed different efficacies, and it is not valid to compare potencies of agonists differing in efficacy (Furchgott, 1972). We therefore determined the dissociation constants for these agonists since comparison of dissociation constants represents a legitimate method of comparing compounds with differing efficacies. The differences in affinity observed for the compounds in rat and rabbit aortae were striking, both in terms of absolute affinities and in the rank order of affinities. In fact, the most potent imidazoline in rat aorta (clonidine) was the weakest in rabbit aorta, while the most potent imidazoline in rabbit aorta (oxymetazoline) was the weakest in rat aorta. The rank order of affinities for the compounds was exactly opposite for rat and rabbit aortae.

In addition, relative efficacies would appear to be another useful manner in which receptors may be subclassified. Large differences in relative efficacies were observed for the series of compounds studied in rat and rabbit aortae. The differences were particularly large for oxymetazoline and tetrahydrozoline. Furthermore, the rank order for relative efficacies was different between the two tissues also indicating that the α-receptors in these tissues are different.

The rat and rabbit aortae are both tissues often used to evaluate postsynaptic α-adrenergic effects. The present investigation and previous studies (Ruffolo *et al.*, 1980b; 1981a; 1982; Randriantsoa *et al.*, 1981) show how important it is not to equate the α-receptor in these two tissues. It is clear that the postsynaptic α-adrenoceptor of rabbit aorta is α_1 (Docherty *et al.*, 1981; Ruffolo *et al.*, 1982). The rat aorta may not be classified as either α_1 or α_2 and may represent yet another α-receptor subtype (Randriantsoa *et al.*, 1981; Ruffolo *et al.*, 1982). Until the nature and significance of the postsynaptic α-adrenoceptor of rat aorta is more clearly understood, results from this tissue should be interpreted with caution.

This is Contribution No. X to Receptor Interactions of Imidazolines.

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