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Heterogeneity of Postsynaptic *Alpha* Adrenergic Receptors in Mammalian Aortas

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ABSTRACT

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Clonidine and yohimbine were used to differentiate postsynaptic *alpha* adrenergic receptors in aortas from six mammalian species. Three distinct postsynaptic *alpha* adrenergic receptor subtypes were observed based on the affinities of clonidine and yohimbine. Receptors with high affinity for both compounds were observed in rat aorta, whereas *alpha* receptors with low affinity were observed in rabbit and guinea-pig aortas. The range of affinities between the *alpha* adrenergic receptor in rat aorta and the corresponding receptor in rabbit and guinea-pig aortas was approximately 170-fold for clonidine and 30-fold for yohimbine. Receptors with intermediate affinity for both clonidine and yohimbine were observed in aortas from hamster, dog and cat. Based on our previous studies in which clonidine and yohimbine also distinguished three types of postsynaptic *alpha* adrenergic receptors in various tissues from the rat, it appears that the *alpha* adrenergic receptor of guinea-pig and rabbit aortas resembles that found in the rat vas deferens, whereas the postsynaptic *alpha* adrenergic receptor in hamster, cat and dog aortas most closely resembles that found in rat spleen, bladder and portal vein. The *alpha* adrenergic receptor of rat aorta which has extremely high affinity for both clonidine and yohimbine is atypical of postsynaptic *alpha* adrenergic receptors found in aortas of other species or in different tissues from the rat. The *alpha* receptor of rat aorta has properties of *both alpha*-1 and *alpha*-2 adrenergic receptors, in contrast to postsynaptic *alpha* receptors in other tissues which resemble only *alpha*-1 receptors. These results indicate that it may not be possible to classify the *alpha* adrenergic receptor of rat aorta using current methods of subclassification (*i.e., alpha*-1 *vs. alpha*-2) and that more than two types of *alpha* adrenergic receptors may exist.

We recently reported that clonidine (Ruffolo *et al.*, 1980b) and yohimbine (Ruffolo *et al.*, 1981a), an *alpha*-2 selective agonist and antagonist, respectively (Starke and Docherty, 1980), can differentiate postsynaptic *alpha* adrenergic receptors in various tissues from the rat into three distinct subtypes. The *alpha* receptor in rat aorta had the highest affinity for both clonidine and yohimbine, whereas the lowest affinities were observed in the vas deferens. The affinities of these compounds in the portal vein, bladder and spleen were between those of the rat aorta and vas deferens.

The unexpectedly high affinities of clonidine and yohimbine in rat aorta were interesting in view of our recent structure-activity studies of imidazolines (Ruffolo *et al.*, 1979a,b,c; 1980a,c) in which large discrepancies were noted between rat aorta and other tissues such as rabbit aorta (Sanders *et al.*, 1975) which contains the type of classical postsynaptic *alpha* adrenergic receptor which is now referred to as an *alpha*-1 adrenergic receptor (Docherty *et al.*, 1981). In many instances, the postsynaptic *alpha* adrenergic receptor of rat aorta resembled the *alpha*-2 adrenergic receptor more than the *alpha*-1 adrenergic receptor (Ruffolo *et al.*, 1981a) and this was also an unexpected observation.

The rat aorta differs from the aortas of other mammalian species in that it lacks a functional adrenergic innervation (Patil *et al.*, 1972). Because sympathetic innervation may play a role in the type and distribution of postsynaptic *alpha* adrenergic receptors in the vasculature (Yamaguchi and Kopin, 1980; Langer *et al.*, 1980) and because our previous results suggested that *alpha* receptors may not be the same in aortas from different species, we decided to investigate the *alpha* adrenergic receptors in aortas from rat, hamster, guinea pig, rabbit, cat and dog. Clonidine and yohimbine were used to detect heterogeneity in postsynaptic *alpha* adrenergic receptors in aortas from these different mammalian species inasmuch as these compounds have proven useful to us previously in detecting *alpha* receptor heterogeneity in various tissues from the rat (Ruffolo *et al.*, 1980b; 1981a).

Methods

General considerations. Male albino rats (Harlan Wistar, 250–425 g), male albino guinea pigs (Murphy, 300–420 g), male albino rabbits

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ABBREVIATION: PSS, physiological salt solution.

(Langshaw, 2–2.5 kg) and male golden hamsters (Harlan, 145–190 g) were sacrificed by a sharp blow to the head. Mongrel dogs (17.5–19 kg) and cats (2.5–5.1 kg) of either sex were sacrificed by pentobarbital (35 mg/kg i.v.) and methoxyflurane anesthesia, respectively. Segments of thoracic aorta were removed and dissected free of fat and connective tissue in PSS (pH 7.40) at room temperature. Helically cut strips, approximately 2 mm wide and 30 mm long, were prepared as described by Furchgott and Bhadrakom (1953). Aortic strips were suspended in 10-ml organ baths containing PSS maintained at 37.5°C and were aerated with a 5% CO_2-95% oxygen mixture. The composition of PSS was (millimolar): 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 10^{-5} M cocaine and 10^{-6} M propranolol to inhibit neuronal uptake and block *beta* adrenergic receptors, respectively. The tissues were attached to a Grass FT-03 isometric transducer connected to a Grass model 7 Polygraph recorder and were allowed to equilibrate under appropriate resting tensions (rat, guinea pig, hamster, 2 g; rabbit, cat, 5g; and dog 10 g) for at least 2 hr before drug addition.

Dose-response curves. Dose-response curves to norepinephrine and clonidine 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 hr to ensure maximum washout of agonist and to minimize the possibility of receptor desensitization. All responses to clonidine are expressed as a percentage of the norepinephrine maximum which was obtained from a norepinephrine dose-response curve before construction of the clonidine dose-response curve.

Determination of dissociation constants of clonidine. Because clonidine is a partial agonist of *alpha* adrenergic receptors relative to norepinephrine in aortas from most mammalian species studied (see fig. 1), the technique of Waud (1969) for determining dissociation constants of partial agonists was used. A dose-response curve was first constructed for norepinephrine and then for clonidine (see above). The relationship between the dose-response curves and of a strong agonist (norepinephrine) and a partial agonist (clonidine) is described mathematically by the following equation (Waud, 1969):

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

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 (referred to as K_D in table 2) is readily obtained from the linear plot of 1/[A] against 1/[P] by the equation (Waud, 1969):

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

The technique of Waud (1969) may be used when $e_A \gg e_P$ or when $K_A \gg [A]$, where [A] equals the ED_{50} of the full agonist. The ratios of K_A:[A] are known for three of the four tissues in which the technique of Waud (1969) was employed. The ratio of K_A:[A] is 12 in rat aorta (Ruffolo *et al.*, 1979c), approximately 16 to 18 in rabbit aorta (Besse and Furchgott, 1976, calculated) and 18 in guinea-pig aorta (R. R. Ruffolo and J. E. Waddell, manuscript in preparation), indicating that the technique of Waud (1969) is appropriate in these tissues. The hamster aorta has not been studied previously in great detail and no such ratio is known. However, since the E_{max} of clonidine (table 1) is the same in hamster aorta relative to the rat, guinea-pig and rabbit aortas, one may assume, by inference, that the ratio of K_A:[A] is of similar magnitude in this tissue as in the others and that the technique of Waud (1969) is also applicable in hamster aorta. In our hands, the technique of Waud (1969) gives results for partial agonists identical to those obtained by other procedures (Ruffolo *et al.*, 1979c).

In the cat and dog aortas in which clonidine does not elicit a contractile response, dissociation constants were obtained by using clonidine as a competitive antagonist of norepinephrine and analyzing these results by the technique of Arunlakshana and Schild (1959) (see below; Ruffolo *et al.*, 1980a). Four concentrations of clonidine ranging from 10^{-6} to 10^{-4} M were tested in each tissue. At least 12 points were obtained for each Schild plot. The slope of the Schild plot for clonidine was 0.94 in the dog aorta and 0.97 in cat aorta. As stated above, we have demonstrated previously that these different methods used for obtaining dissociation constants yield results that are not significantly different (Ruffolo *et al.*, 1979c).

Determination of dissociation constants for yohimbine. Dissociation constants (K_D) of yohimbine were determined by the technique of Arunlakshana and Schild (1959) using norepinephrine as the agonist. A dose-response curve to norepinephrine was first constructed as described above. After thorough washout of the agonist, the tissues were incubated with yohimbine (10^{-7}–10^{-4} M) for 60 min. In the presence of yohimbine, a second dose-response curve to norepinephrine was constructed. Dose ratios (*ie*, ED_{50} of norepinephrine in the presence of yohimbine divided by the control norepinephrine ED_{50}) were determined at different concentrations of yohimbine. According to Arunlak-

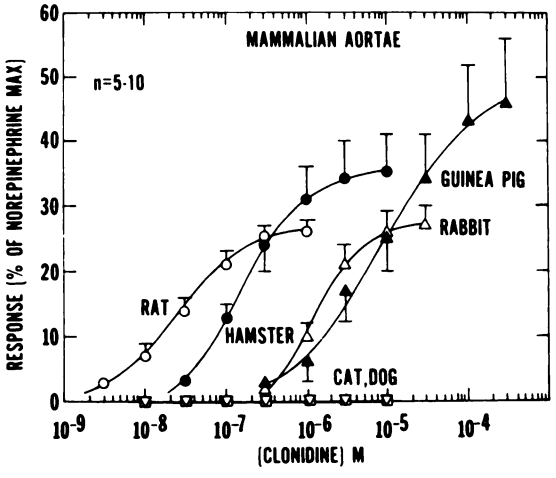

Fig. 1. Dose-response curves for clonidine in aortas from six mammalian species. Each point is the mean ± S.E.M. of 5 to 10 observations. All responses are expressed as a percentage of the maximum response to norepinephrine which was obtained in all tissues before construction of the clonidine dose-response curve. The symbols correspond to the aortas of the various species as follows: ○, rat; ●, hamster; △, rabbit, ▲, guinea pig; ▽, cat; and □, dog.

TABLE 1

Important characteristics of dose-response curves to clonidine and norepinephrine in aortas from various mammalian species

Species	*n*	Clonidine		Norepinephrine
		E_{max}[a]	−log ED_{50}[b]	−log ED_{50}
Rat	7	0.26 ± 0.02	7.62 ± 0.02	7.85 ± 0.06
Hamster	5	0.35 ± 0.06	6.82 ± 0.06	7.46 ± 0.05
Rabbit	5	0.27 ± 0.03	5.76 ± 0.07	6.97 ± 0.04
Guinea pig	5	0.46 ± 0.10	5.27 ± 0.17	6.02 ± 0.06
Cat	10	0	N.A.[c]	6.33 ± 0.12
Dog	10	0	N.A.[c]	6.18 ± 0.11

[a] Maximum contractile response of clonidine relative to norepinephrine. N.A., not applicable.

[b] Mean ± S.E.M.

[c] −log ED_{50} could not be determined because clonidine failed to elicit a contractile response.

shana and Schild (1959), if blockade is competitive, a plot of the logarithm of (dose ratio −1) against the negative logarithm of the molar concentration of antagonist should produce a straight line with a slope of 1 and intercept along the abscissa of $-\log K_D$. In all experiments, at least one tissue was run in parallel with the experimental tissues, but received no antagonist, and was used to correct for time-dependent changes in agonist sensitivity (Furchgott, 1972).

The analyses described above for determining the dissociation constants of partial agonists (clonidine) by the technique of Waud (1969) and competitive antagonists (yohimbine in all tissues and clonidine in cat and dog aortas) by the technique of Arunlakshana and Schild (1959) in isolated smooth muscle preparations were performed with the aid of a computer and digital plotter by previously published procedures (Zaborowsky *et al.*, 1980).

Statistical evaluation. The results are expressed as the mean ± S.E.M. Statistical differences between two means ($P < .05$) were determined by Student's *t* test for unpaired observations or by testing for overlap of 95% confidence limits (Sokal and 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 and Rohlf, 1969). The slopes of regression lines were tested for significance by an *F* test (Woolf, 1968).

Drugs. All drug solutions were prepared daily in demineralized water or saline. (−)-Norepineprhine·HCl was purchased from Sterling-Winthrop Research Institute (Rensselaer, NY) and yohimbine was purchased from Sigma Chemical Company (St. Louis, MO). Clonidine was generously supplied by Boehringer-Ingelheim Ltd. (Elmsford, NY).

Results

Dose-response curves to clonidine in aortas from six mammalian species are presented in figure 1. Because all experiments were performed in the presence of 10^{-5} M cocaine and 10^{-6} M propranolol, it is assumed that all observed responses are the result of direct postjunctional *alpha* adrenergic receptor activation. It is apparent from figure 1 that clonidine is a partial agonist relative to norepinephrine in aortas from rat, hamster, guinea pig and rabbit. At no dose up to 10^{-5} M did clonidine evoke a contractile response in dog or cat aorta. The $-\log ED_{50}$ values and maximum contractile effects (E_{max}) for clonidine and norepinephrine in these tissues are presented in table 1. The ED_{50} values of clonidine span a range of over 200-fold.

The wide variation of ED_{50} values for clonidine suggests *alpha* receptor heterogeneity. However, the use of ED_{50} values in receptor subclassification is tenuous due to differences among tissues in receptor reserves and thresholds (Furchgott, 1972). In addition, ED_{50} values cannot be obtained for those compounds that do not elicit an effect in a given tissue (*i. e.*, clonidine in cat and dog aortas), but will nevertheless still bind with high affinity to the receptor in that tissue. To circumvent these potential problems, dissociation constants for clonidine were determined in all tissues. Comparison of dissociation constants of both agonists and antagonists is one of the most reliable techniques used to differentiate receptors and receptor subtypes (Furchgott, 1972). The dissociation constants of clonidine in aortas from different species are listed in table 2 and are presented with 95% confidence limits in figure 2. Statistically significant differences ($P < .05$) between receptor subtypes exist when 95% confidence limits do not overlap. It is clear from figure 2 that clonidine can differentiate postsynaptic *alpha* adrenergic receptors in these aortas into three different classes. Clonidine has highest affinity for those *alpha* receptors located in rat aorta and lowest affinity for the receptors in guinea-pig and rabbit aortas. The difference in affinity for clonidine between rat and guinea-pig aorta is 174-fold. The affinity of clonidine for the *alpha* adrenergic receptors of hamster, cat and dog aortas is intermediate between that of rat aorta and guinea-pig (or rabbit) aorta.

Schild plots for yohimbine used as a competitive antagonist of norepinephrine in aortas from these six species are presented in figure 3. In all aortas, blockade by yohimbine was in fact shown to be competitive as evidenced by the linearity of the Schild plots and the excellent agreement of the calculated slopes with the expected theoretical value of unity (table 3).

TABLE 2

Dissociation constants[a] and relative affinities of clonidine in aortas from various mammalian species

Species	*n*	$-\log K_D$[b]	Relative Affinity
Rat	7	7.41 ± 0.09	174
Hamster	5	6.39 ± 0.16	17
Dog	15	6.39 ± 0.09	17
Cat	15	6.28 ± 0.06	13
Rabbit	5	5.31 ± 0.04	1
Guinea pig	5	5.17 ± 0.04	1

[a] Dissociation constant determined by the technique of Waud (1969) for partial agonists for rat, hamster, rabbit and guinea-pig aortas and by the technique of Arunlakshana and Schild (1959) for cat and dog aortas.
[b] Mean ± S.E.M.

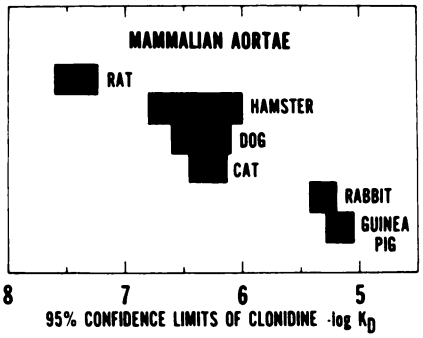

Fig. 2. Mean $-\log K_D$ values and 95% confidence limits for clonidine in aortas from six mammalian species. Overlap of 95% confidence limits indicates that $-\log K_D$ values are not significantly different ($P > .05$).

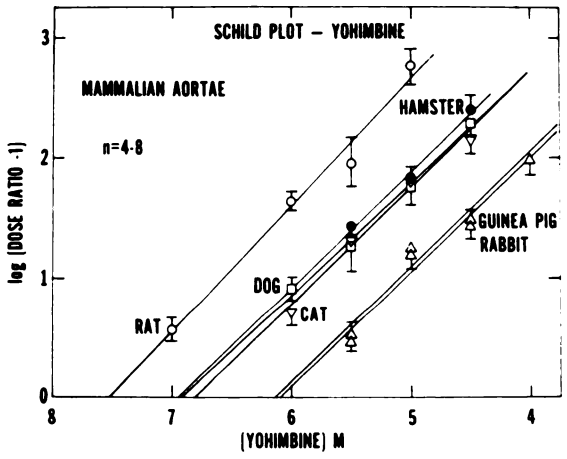

Fig. 3. Schild plots for yohimbine as a competitive antagonist of norepinephrine in aortas from six mammalian species. Each point is the mean ± S.E.M. of at least four observations. The intercept of each line along the abscissa is the $-\log K_D$. The slopes of each line are not significantly different ($P > .05$) from the theoretical value of unity. See legend to figure 1 for description of symbols.

The $-\log K_D$ values calculated from these Schild plots are listed in table 3 and are presented with 95% confidence limits in figure 4. The $-\log K_D$ values for yohimbine fall into three significantly different ($P < .05$) groups which are identical to the three groups observed with clonidine (fig. 2). Postsynaptic *alpha* adrenergic receptors with high affinity for yohimbine were observed in rat aorta, whereas *alpha* receptors with low affinity for yohimbine were observed in both rabbit and guinea-pig aortas. The difference in affinity between these two classes of postsynaptic *alpha* receptors which represent the extremes is approximately 30-fold. The postsynaptic *alpha* adrenergic receptors of hamster, cat and dog aortas were intermediate in affinity for yohimbine and were not significantly different from one another. The $-\log K_D$ value of 6.04 obtained for yohimbine in rabbit aorta is different from the value of approximately 6.7 obtained by Furchgott (1955) and Sheys and Green (1972). The reason for this discrepancy is not clear but may owe, in part, to the fact that the present studies were performed after uptake-1 and *beta* adrenergic receptors were inhibited.

Discussion

We previously have used clonidine (Ruffolo *et al.*, 1980b) and yohimbine (Ruffolo *et al.*, 1981a), both being selective for *alpha* 2 adrenergic receptors (Starke and Docherty, 1980), to differentiate three subtypes of postsynaptic *alpha* adrenergic receptors in tissues from the rat. Likewise, Barker *et al.* (1977) were able to demonstrate three distinct categories of postsynaptic *alpha* adrenergic receptors in a variety of tissues from several rodent species using the technique of isomeric activity ratios developed by Patil *et al.* (1971), whereas Sheys and Green (1972) were able to distinguish at least two types in rabbit spleen and aorta using a series of agonists and antagonists.

TABLE 3

Important characteristics of Schild plots of yohimbine against norepinephrine in aortas from different mammalian species

Species	n[a]	Slope[b]	$-\log K_D$[c]	Relative Affinity[d]
Rat	23	1.05	7.51 ± 0.07	30
Hamster	12	0.97	6.94 ± 0.08	8
Dog	16	0.93	6.93 ± 0.09	8
Cat	16	0.97	6.79 ± 0.10	6
Rabbit	30	0.94	6.04 ± 0.04	1
Guinea pig	19	0.95	6.12 ± 0.04	1

[a] *n* refers to the sum of all values utilized in the Schild plot.
[b] Slope of Schild plot. Theoretical value equals unity for competitive antagonist.
[c] Mean ± S.E.M.
[d] Affinity relative to rabbit aorta.

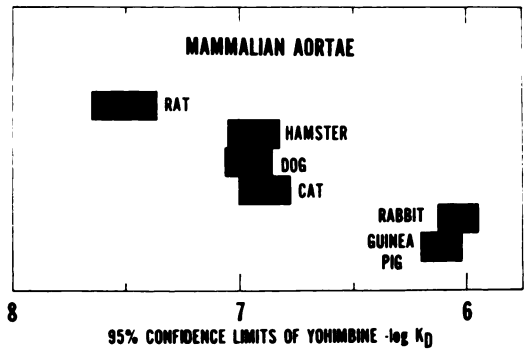

Fig. 4. Mean $-\log K_D$ values and 95% confidence limits for yohimbine in aortas from six mammalian species.

Harper *et al.* (1979) have reported heterogeneity in postsynaptic *alpha* adrenergic receptors in rat, guinea-pig and rabbit spleen. In the present study, postsynaptic *alpha* adrenergic receptors in aortas from six mammalian species could be divided into three statistically different groups using clonidine and yohimbine, both compounds being selective for *alpha*-2 adrenergic receptors.

As in our previous studies, the postsynaptic *alpha* adrenergic receptor of rat aorta possessed the highest affinity for both clonidine and yohimbine. The $-\log K_D$ values of 7.41 and 7.51 for clonidine and yohimbine, respectively, obtained in rat aorta in the present investigation are in good agreement with the values of 7.74 and 7.70, respectively, reported by us previously (Ruffolo *et al.*, 1980b, 1981a). Markedly lower affinities for both clonidine and yohimbine were observed in guinea-pig and rabbit aortas. Based on the calculated dissociation constants for clonidine and yohimbine, the postsynaptic *alpha* adrenergic receptor in guinea-pig and rabbit aortas is similar to the *alpha* receptor we previously have characterized in rat vas deferens. It must be emphasized, however, that similar dissociation constants obtained in different tissues for only two agents does not necessarily prove that the receptors are the same. Similar dissociation constants for a number of other agents would be required for conclusive evidence of similarity of receptor subtypes.

The postsynaptic *alpha* adrenergic receptor in aortas from hamster, cat and dog has significantly ($P < .05$) lower affinity for both clonidine and yohimbine than that observed in rat aorta, yet significantly higher affinity for both compounds than that observed in rabbit and guinea-pig aortas. The *alpha* adrenergic receptor of hamster, cat and dog aortas with "intermediate" affinities for clonidine and yohimbine closely resembles the class of postsynaptic *alpha* adrenergic receptors in rat spleen, portal vein and bladder which we have shown also to possess an intermediate affinity for both clonidine and yohimbine (Ruffolo *et al.*, 1980b, 1981a).

The affinities of clonidine and yohimbine for what we have termed both the intermediate affinity receptors (*ie*, hamster, cat and dog aortas and rat portal vein, spleen and bladder) and "low" affinity receptors (*ie*, rabbit and guinea-pig aortas and rat vas deferens) are all within the range of affinities of these compounds characteristic of *alpha*-1 adrenergic receptors (Ruffolo *et al.*, 1980b; 1981a). In addition, the alpha-1 selective agonist, phenylephrine (Patil *et al.*, 1972; Sanders *et al.*, 1975; Ruffolo *et al.*, 1980b, 1981a; Ruffolo and Patil, 1979), and the *alpha*-1 selective antagonist, prazosin (Doggrell and Paton, 1978a; U'Prichard *et al.*, 1978; Furchgott, 1980; R. Ruffolo and J. Waddell, unpublished observations), are relatively potent in most of these tissues. Thus, the possibility that these tissues comprise two different subtypes of *alpha*-1 adrenergic receptors must be considered.

Of the various aortas studied in the present investigation, the rat is the only species in which the aorta lacks a functional adrenergic innervation (Patil *et al.*, 1972). The possibility exists that the lack of innervation may be related to the unusual characteristics of the *alpha* adrenergic receptor of rat aorta. Yamaguchi and Kopin (1980) and Langer *et al.* (1980) have speculated that postsynaptic *alpha* adrenergic receptors are of the *alpha*-1 type when they are located close to the sympathetic nerve terminals (junctional) and are of the *alpha*-2 type when they are at a distance from the terminals (extrajunctional). The lack of innervation to the rat aorta would, for all intents and purposes, make the *alpha* receptors in this tissue

"extrajunctional" which may account for their uniqueness among *alpha* receptors in mammalian aortas and may serve to explain, in part, their resemblance to *alpha*-2 receptors (see below).

Rat aorta. The characteristics of the *alpha* adrenergic receptor of rat aorta are somewhat of a dilemma. The *alpha* adrenergic receptor in this tissue has unexpectedly high affinity for the *alpha*-2 selective agonist and antagonist, clonidine and yohimbine, respectively. In fact, the affinities of both clonidine and yohimbine in rat aorta are exactly what one would expect, based on literature values, for *alpha*-2 adrenergic receptors (see Ruffolo *et al.*, 1980b, 1981a). In addition, most of the structure-activity relationships we have published for imidazolines in rat aorta have tended to be characteristic of *alpha*-2 adrenergic receptors rather than *alpha*-1 (see below). This led us previously to classify the postsynaptic *alpha* adrenergic receptor in rat aorta as *alpha*-2 (Ruffolo *et al.*, 1981a). In addition to the evidence cited above, the literature contains a good deal of evidence to support this classification. According to the method of *alpha* receptor subclassification recently developed by Wikberg (1978), when the potency of phenylephrine > clonidine, the receptor is *alpha*-1 and when clonidine > phenylephrine, the receptor is *alpha*-2. In rat aorta, when using either ED_{50} values or dissociation constants for comparison, clonidine is always more potent than phenylephrine (Ruffolo *et al.*, 1979c), suggesting the existence of *alpha*-2 receptors. Furthermore, Drew (1981) has shown that the phenethanolamine-N-methyltransferase inhibitor SKF 64139 is a selective *alpha*-2 antagonist having a $-\log K_D$ of 6.79 for *alpha*-2 receptors of guinea-pig ileum and 5.47 for *alpha*-1 receptors of rabbit aorta. In rat aorta, we have reported that the $-\log K_D$ of SKF 64139 is 6.52 (Fuller *et al.*, 1981), a value nearly identical to that of Drew (1981) for *alpha*-2 adrenergic receptors.

Timmermans and van Zwieten (1977) published a detailed structure-activity relationship for the centrally mediated antihypertensive actions of imidazolines structurally related to clonidine. This activity is proposed to be mediated *via* central *alpha*-2 adrenergic receptors (Timmermans *et al.*, 1980). The structure-activity relationships involving the *alpha*-2 selective compounds studied both by Timmermans and van Zwieten (1977) and by us in rat aorta (Ruffolo *et al.*, 1980a) were remarkably similar ($r = 0.90$, $P < .05$), again suggesting the presence of *alpha*-2 receptors in rat aorta. To carry the analogy further, we have reported recently that the rat aorta will predict the *alpha*-2 effects of drugs at the vasomotor center of spontaneously hypertensive rats (Ruffolo *et al.*, 1981b) in which it was shown that the centrally mediated depressor effects of a series of mono- and dimethoxy-substituted tolazoline derivatives were highly correlated with the relative efficacy values obtained in rat aorta. These results might also be interpreted as evidence for the existence of *alpha*-2 adrenergic receptors in the rat aorta.

As stated earlier, the structure-activity relationships of imidazolines published by us in rat aorta most closely correlate with the *alpha*-2 adrenergic effects of these imidazolines and not the *alpha*-1 effects. This is evident from figure 5 in which a series of compounds studied by us in rat aorta (Ruffolo *et al.*, 1979c, 1980a, 1981a) and by many other groups (see figure legend) in *alpha*-1 and *alpha*-2 test systems are compared. In this comparison, care was taken to use dissociation constants wherever possible and to use ED_{50} values only when no other data were available. In all cases, comparisons of dissociation constants in other systems were made with dissociation constants in rat aorta and, likewise, ED_{50} values were compared to ED_{50} values. For those compounds (see legend) studied both by us and by others, the results from rat aorta were significantly correlated ($P < .01$) only with the *alpha*-2 adrenergic effects and *not* the *alpha*-1 effects, again showing the similarity of the *alpha* adrenergic receptor in rat aorta to the *alpha*-2 subtype.

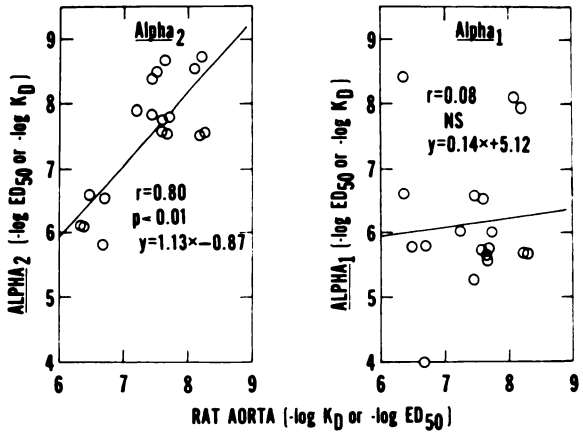

Fig. 5. Correlation between $-\log K_D$ and/or $-\log ED_{50}$ values obtained in rat aorta with analogous values from the literature in which *alpha*-1 and *alpha*-2 adrenergic tests systems were used. The compounds in the correlations are: clonidine, naphazoline, oxymetazoline, phentolamine, yohimbine, tolazoline, ST600, ST91, xylometazoline, phenylephrine, phenyliminoimidazoline and dihydroxyphenyliminoimidazoline. The literature values for *alpha*-1 and *alpha*-2 adrenergic receptors were obtained from the following references: Jarrott *et al.* (1979a, b), Sanders *et al.* (1975), Drew (1978), Malta *et al.* (1980) and Hieble and Pendleton (1979). The values in rat aorta were obtained from: Ruffolo *et al.* (1979c, 1980a, 1981a).

In spite of this evidence in which the *alpha* receptor of rat aorta is shown to resemble closely the *alpha*-2 subtype, it is now known that the highly selective *alpha*-1 agonists, phenylephrine and methoxamine, and the *alpha*-1 antagonist, prazosin, are also potent in rat aorta (Doggrell and Paton, 1978b; Ruffolo *et al.*, 1979c; Downing *et al.*, 1981) making our previous subclassification of this receptor as *alpha*-2 highly suspect. At the present time, it is not possible to resolve this apparent discrepancy. However, one must conclude that the *alpha* adrenergic receptor of rat aorta possesses properties of *both alpha*-1 and *alpha*-2 adrenergic receptors. As such, the receptor in rat aorta is *different* from the *alpha*-1 and *alpha*-2 receptors in other tissues and therefore should not be forced into the *alpha*-1/*alpha*-2 type of subclassification. This conclusion is consistent with that of Downing *et al.* (1981) who have shown that the separate components in the biphasic contractile response of rat aorta to *alpha* adrenergic agonists could not be ascribed to *either alpha*-1 or *alpha*-2 stimulation. Recently, Randriantsoa *et al.* (1981) presented evidence suggesting that the *alpha* receptor of rat aorta could not be simply classified as either *alpha*-1 or *alpha*-2, and that another *alpha* receptor subtype may also exist in this tissue. Comparison of our results in rat aorta with those reported by others for the atypical postsynaptic *alpha* adrenergic receptor in dog saphenous vein (Sullivan and Drew, 1980; De Mey and Vanhoutte, 1981) indicate that these two unusual *alpha* adrenergic receptors may be similar.

The results of this study may be summarized as follows: 1) postsynaptic *alpha* adrenergic receptors in aortas from six mammalian species may be divided into three distinct classes by clonidine and yohimbine; 2) these three classes appear similar to those differentiated previously by us (Ruffolo *et al.*, 1980b, 1981a) for postsynaptic *alpha* receptors in various pe-

ripheral tissues of the rat; 3) the *alpha* adrenergic receptor of rat aorta (but not aortas from other species) has high affinity for clonidine and yohimbine and has many other characteristics expected for *alpha*-2 adrenergic receptors; 4) results to date indicate that the postsynaptic *alpha* adrenergic receptor of rat aorta has characteristics of *both alpha*-1 and *alpha*-2 adrenergic receptors, but appears to be different from either of these two receptor subtypes; and 5) the *alpha* adrenergic receptor of rat aorta cannot (and should not) be forced into the classification of either *alpha*-1 or *alpha*-2.

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