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Characterization of the *Alpha*-1 Adrenergic Receptors in the Thoracic Aorta of Control and Aldosterone Hypertensive Rats: Correlation of Radioligand Binding with Potassium Efflux and Contraction[1]

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ABSTRACT

Pharmacologic analysis of functional and radioligand binding studies were used to determine whether alterations in adrenergic receptors contribute to the catecholamine supersensitivity observed in the thoracic aorta of aldosterone hypertensive rats (AHR). Adrenergic function was evaluated using receptor-mediated contraction and rate of ^{42}K efflux. The pA_2 for phentolamine was the same in AHR (7.9 ± 0.1 and 8.0 ± 0.1) and control (7.9 ± 0.2 and 8.1 ± 0.1) rats whether measured by contraction or ^{42}K efflux. The pA_2 value for the selective *alpha*-1 antagonist prazosin (9.8 ± 0.1 to 10.7 ± 0.2) and the *alpha*-2 antagonist yohimbine (6.6 ± 0.2 to 7.4 ± 0.2) was similar in AHR and control groups using both norepinephrine and phenylephrine as agonists. The rank order of potency was prasozin > phentolamine > yohimbine in both groups. The K_D for [^{3}H]prazosin binding to AHR (26 ± 3 pM) and control tissue (34 ± 6 pM) agreed with the prazosin K_B obtained by measurements of contraction and ^{42}K efflux. The *alpha*-1 receptor density was also unaltered: 39 ± 1 fmol/mg in AHR and 44 ± 3 fmol/mg in control. Assessment of the NE dissociation constant (K_a) by method of fractional receptor inactivation indicated that the K_a was 4.8×10^{-7} M in both AHR and control tissues. However, at the same receptor occupancy, the NE-induced increase in ^{42}K efflux was elevated markedly in aorta from AHR. We conclude that alteration in adrenergic receptor density, affinity or type is not the cause of catecholamine supersensitivity in the aorta from AHR and that postreceptor events mediate this phenomenon.

Catecholamine supersensitivity, a common feature in several models of experimental hypertension (Garwitz and Jones, 1982; Holloway and Bohr, 1973; Jones, 1973), precedes the elevation of blood pressure (Berecek *et al.*, 1980; Garwitz and Jones, 1982; Katovich *et al.*, 1984) and therefore may be an important contributor to the pathogenesis of increased peripheral resistance. Alterations leading to the development of supersensitivity involve the postjunctional vascular smooth muscle as neither innervation or structural changes (Friedman, 1983) are required for its manifestation. An increase in the passive flux of monovalent ions occurs in the arteries from hypertensive rats (Garwitz and Jones, 1982; Jones, 1973; Friedman and Friedman, 1976) which has led to the proposal that an increase in membrane permeability of the vascular smooth muscle plays a role in the altered responsiveness to catecholamines. However, in the AHR the development of supersensitivity to catecholamines and the increase in vascular permeability are not parallel (Garwitz and Jones, 1982) and the dissociation of these two events suggests that other mechanisms may be involved. One alternative hypothesis is that an alteration occurs in the adrenergic receptor located on the postjunctional membrane which mediates catecholamine-induced vasoconstriction and therefore influences directly vascular resistance. A variety of physiologic as well as pathologic conditions can modulate the number or affinity of the adrenergic receptor (Colucci *et al.*, 1984; Lefkowitz, 1982; Motulsky and Insel, 1982) and changes in either parameter could result in increased responsiveness to catecholamines. It is also possible that the receptor type which mediates agonist-induced vasoconstriction in rat aorta is altered.

The purpose of this study was to determine whether changes occur in *alpha* adrenergic receptor affinity, number or subtype in vascular smooth muscle from AHR. Measures of the inhibitory action of both competitive and noncompetitive antagonists were made on adrenergic activation of contraction and ^{42}K efflux. These pharmacologic measurements of receptor affinity for both agonists and antagonists were correlated with radioligand binding studies which, in addition, provided information

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ABBREVIATIONS: AHR, aldosterone hypertensive rat; NE, norepinephrine; PE, phenylephrine; Db, dibenamine; [^{125}I]HEAT, β-3-iodo-[^{125}I]-4-hydroxyphenyl-ethyl-aminomethyl-tetralone; B_{max}, maximum binding.

on receptor density. Preliminary findings from this study have been reported previously (Jones *et al.*, 1983).

Methods

Animals and tissue preparation. The aldosterone hypertensive rat model has been described previously (Garwitz and Jones, 1982). The left kidney was removed from anesthetized male Sprague-Dawley rats (150–175 g), an osmotic minipump (Alza Corp., Palo Alto, CA) implanted s.c. and the animals were given 1% NaCl (supplemented with 0.3% KCl to maintain K^+ balance in rats which received aldosterone) to drink. The *d*-aldosterone (Sigma Chemical Co., St. Louis, MO) was dissolved in polyethylene glycol and was infused at a rate of 1.0 $\mu g \cdot hr^{-1}$ for a minimum of 2 weeks, which produced a significant elevation in systolic blood pressure determined by the tail-cuff method. Each AHR was required to have a minimum systolic blood pressure of 180 mm Hg. The average systolic pressure of control-salt animals was 120 mm Hg. Control animals used in initial experiments were nephrectomized, infused with polyethylene glycol *via* the osmotic minipump and given 1% NaCl to drink. However, in subsequent experiments the pump was not implanted as vehicle infusion did not produce any detectable differences in the control group (data not shown). A third control group with intact kidneys was given tap water to drink. All animals were given a diet of normal rat chow (Purina).

On the morning of the experiment, the animals were decapitated and the thoracic aorta removed quickly and placed in low calcium (0.25 mM Ca^{++}) potassium-free dissection solution (to reduce endogenous potassium in preparation for ^{42}K equilibration). Loose connective tissue and fat were dissected from the vessel which was then slit lengthwise. After endothelial cells were removed from the strips by lightly stroking the intimal surface with moistened filter paper, the vessel was cut in half and mounted on stainless-steel holders.

Solutions. Normal physiological solution had the following composition in millimolar: Na^+, 146.2; K^+, 5.0; Mg^{++}, 1.2; Ca^{++}, 2.5; Cl^-, 143.9; HCO_3^-, 13.5; H_2PO_4, 1.2; and glucose, 11.4. Solutions were gassed with a 97% O_2-3% CO_2 mixture to obtain a pH of 7.4. Propranolol (3 μM), EDTA (0.1 mM) and ascorbic acid (1 mM) were added to all solutions for *beta* receptor blockade and inhibition of catecholamine oxidation, respectively. Uptake-1 and uptake-2 inhibitors were not included in the solutions, as the rat aorta is sparsely innervated (Burnstock, 1975) and deoxycorticosterone, an uptake-2 inhibitor, does not alter adrenergic stimulation of ^{42}K efflux (Koehler *et al.*, 1979).

Aqueous stock solutions were prepared for NE, PE, angiotensin, Db, yohimbine and phentolamine. Prazosin was dissolved in 70% ethanol. All drugs were obtained from Sigma except phentolamine (Ciba Pharmaceutical Co., Summit, NJ) and prazosin (Pfizer Inc., New York, NY).

Potassium efflux experiments. These procedures have been published previously (Jones, 1973) and evaluated (Jones, 1980). Briefly, the tissue was incubated for 2.5 to 3 hr at 37°C in physiological solution containing ^{42}K (University of Missouri Research Reactor, 20 μCi/ml). For studies with competitive antagonists, one-half of the aorta was moved through a series of tubes containing nonradioactive physiological solution, while the other half of the aorta was exposed throughout the experimental protocol (220 min) to the competitive antagonist. The tissues were exposed periodically to the agonist for 10 min followed by a 20-min wash before exposure to the next higher concentration. Initial experiments suggested that the competitive antagonist prazosin may delay the agonist-induced increase in ^{42}K efflux. Consequently, when prazosin was used, the tissue was exposed to the agonist for 15 min to ensure an accurate estimate of the response.

The gamma spectrophotometer (Packard Instrument Co., Inc., Downers Grove,IL) was used to count ^{42}K activity. Washout curves and rate constants were calculated as reported previously (Garwitz and Jones, 1982) on an IBM personal computer. The rate constants at the 30- to 40-min period were used to determine steady-state turnover for statistical comparisons. Dose-response relations were derived from standard normalizing procedures used for the study of drug supersensitivity (Fleming *et al.*, 1973). The response to a given concentration of agonist, Δk, was the difference between the highest rate constant in the presence of the agonist (which generally was the initial phasic response in the first 2 min) and the rate constant for the preceding wash period. For each tissue, the individual responses, Δk were normalized in terms of Δk_{max} (the response to supramaximal concentration of agonist) and represented as a percentage. The median effective concentration, EC_{50}, was determined for each tissue by linear interpolation between the log dose just below and just above the 50% response. The antagonist dissociation constant, K_B, was derived by means of the following equation (based on occupational theory of drug-receptor interaction):

$$K_B = \frac{[B]}{\text{dose ratio} - 1}$$

where dose ratio is the shift in EC_{50} due to the antagonist and B is the concentration of antagonist. Experiments determined that there was no difference in K_B whether the ^{42}K efflux was measured during the peak response or at steady state (10–15 min).

Noncompetitive antagonist. The dissociation constant, K_e, for NE-*alpha* receptor complex was determined by means of the method developed by Furchgott (1966). The procedure involves the analysis of dose-response data obtained with the agonist before and after irreversible inactivation of a fraction of receptors by a noncompetitive antagonist, Db.

After 2 hr of incubation in ^{42}K, one-half of each control and hypertensive aorta was placed in a separate isotopic solution containing Db. The Db had been acidified with HCl (final concentration, 0.01 N HCl) and added to ^{42}K-Krebs' solution for 5 min to allow sufficient time for formation of the reactive ethylenimmonium intermediate. The binding of Db to receptors is both time and concentration dependent (Furchgott, 1966). Pilot experiments determined that a 15-min incubation with Db, 2.0×10^{-7} M for control and 5.0×10^{-7} M for hypertensive tissue, would decrease the maximal response by approximately 50%, a reduction suitable for K_e determination (Furchgott, 1966). At the end of the 15-min incubation period, the tissue was washed in physiological solution for 15 min to prevent any additional inactivation and then returned to the initial ^{42}K solution to complete the 3-hr equilibration. As described above, the tissues were then passed through tubes containing nonradioactive Krebs', exposed to stepwise increases in NE and the subsequent change in ^{42}K efflux was calculated.

To calculate K_e for each experiment, the concentration of NE which caused an equivalent change in ^{42}K efflux after Db treatment (A′) as before Db treatment (A) was determined from the intermediate responses of the dose-response curve for NE *vs.* ^{42}K efflux. A plot of the reciprocal of these concentrations (1/A and 1/A′) was then constructed with a minimum of three points and the K_e was determined from the slope and intercept (Furchgott, 1966).

Contraction experiments. The thoracic aorta was placed in low Ca^{++}, K-free dissection solution and cleaned of fat and loose connective tissue similarly to those used for efflux studies. The aorta was then cut into rings (3.5 mm wide, 0.14 mm thick) and the endothelium was removed by lightly stroking the intimal layer with filter paper moistened with dissection solution. The rings were stretched 1⅓ times their resting diameter by means of a micrometer mounted on a force transducer. The tissue was then placed in vigorously gassed physiological solution maintained at 37°C and equilibrated for 1 hr. Successively higher concentrations of the agonist were then added cumulatively to the bath. The tissue was exposed to each concentration of the agonist until the contractile response reached a plateau which usually occurred in 10 min. The tension recorded during this tonic response was used in the calculation of EC_{50}. After a 45-min wash in physiological solution, the tissue was exposed to the antagonist for a minimum of 20 min (pilot studies indicated that 20- and 90-min incubation produced identical results) and the addition of agonist was repeated in the presence of antagonist. The EC_{50} was determined for each dose response curve and the K_B was calculated as described above for efflux experiments. A

correction factor was incorporated into the estimation of dose ratios and K_B for control-salt and control-H_2O groups because the second dose-response curve shifted to the right with time. Dose-response curves in AHR were superimposable.

Radioligand binding experiments. Adipose and connective tissue were dissected from the thoracic aorta, endothelial cells removed and the aorta finely minced (McIlwain chopper) at 2°C. The minced tissue was homogenized with a Brinkman Polytron (setting no. 7) for two 20-sec bursts and centrifuged at 49,000 × g for 10 min. The resulting pellet was homogenized in a similar manner and filtered through 53-μ nylon mesh to remove elastin and large fragments from the preparation. The filtered homogenate was again centrifuged at 49,000 × g for 10 min. The aortas from approximately three ([^{125}I]HEAT) or nine ([^{3}H]prazosin) rats were used for each binding curve. In saturation binding studies with [^{125}I]HEAT, total binding was determined by incubating in triplicate 970 μl of a crude membrane particulate fraction in 25 mM glycylglycine buffer, pH 7.6, plus 20 μl of various concentrations of [^{125}I]HEAT, (specific activity, 2200 Ci/mmol) diluted in 5 mM HCl. A parallel set of tubes was processed in duplicate with 100 μM (−)-NE to determine nonspecific binding. Specific binding was calculated as the difference between total and nonspecific binding. Saturation experiments with [^{3}H]prazosin (specific activity, 81 Ci/mmol) and [^{3}H] yohimbine (specific activity, 82.7 Ci/mmol) were done in a similar manner except the final assay volume in the prazosin experiments was 2.0 ml. The final protein concentrations in the saturation experiments were 0.02 to 0.06 mg/ml for [^{125}I]HEAT, 0.04 to 0.07 mg/ml for [^{3}H] prazosin and 0.4 mg/ml for [^{3}H]yohimbine. After a 45 [^{125}I]-HEAT) or 30 min ([^{3}H]prazosin; [^{3}H]yohimbine) incubation at 23°C, the suspensions were filtered through GF/B glass fiber filter paper presoaked in 0.1% polyethylenimine using a Brandel cell harvester. The tubes and filters were washed twice with 5 ml of 50 mM Tris-HCl buffer and the radioactivity retained on the filter paper was determined by scintillation spectroscopy with an efficiency of 77 and 41% for ^{125}I and ^{3}H, respectively. Protein concentrations were determined by the method of Lowry *et al.* (1951) using bovine serum albumin as the standard. [^{125}I] HEAT, [^{3}H]prazosin and [^{3}H]yohimbine were purchased from New England Nuclear (Boston, MA).

Inhibition experiments were done by incubating the tissue preparation with 8 to 11 concentrations of nonradiolabeled drug in duplicate and a fixed concentration of radioligand. Mean inhibitory concentration (IC_{50}) values were determined by log-logit analysis.

Statistics. It has been reported previously that the equieffective concentration of NE in a variety of experimental preparations are normally distributed on a log rather than arithmetic scale (Fleming *et al.*, 1972). In these studies, the frequency distribution of the EC_{50} for NE-induced increase in ^{42}K efflux was evaluated on a logarithmic and arithmetic basis; only the log approximates the normal distribution. Therefore, log values were used to make statistical comparison of the EC_{50}. Significance at the level of $P < .05$ was determined using the Student's t test.

Results

Competitive Antagonists

Phentolamine. The *alpha* adrenergic receptor in control-salt and AHR tissues was studied initially by evaluating the effect of phentolamine, a nonsubtype selective competitive antagonist, on the NE-induced increase in ^{42}K efflux (fig. 1). The basal ^{42}K efflux in control aorta was 0.0098 ± 0.0004, the Δk_{max} was 0.0109 ± 0.0007 and the NE EC_{50} was 35 ± 12 nM. The addition of 1 μM phentolamine did not alter the basal ^{42}K efflux (0.0094 ± 0.0002) or Δk_{max} (0.0105 ± 0.0011), but shifted the NE EC_{50} to 4.7 ± 2.0 μM ($P < .001$), a dose ratio of 163. Aldosterone-treated rats had a significantly elevated basal efflux (0.0183 ± 0.0010, $P < .001$), a similar Δk_{max} (0.0128 ± 0.0040) and an increased sensitivity to NE, reflected in the lower NE EC_{50} of 2.4 ± 0.5 nM ($P < .001$). These differences

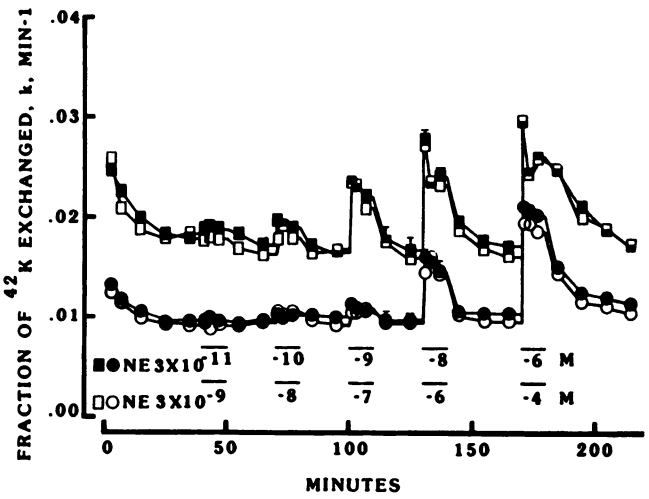

Fig. 1. Effects of NE on aortic ^{42}K turnover from aldosterone-treated (■, □; $n = 7$) and control-salt (●, ○; $n = 7$) rats. One-half of each aorta was exposed to NE alone (■, ●) and the other half was exposed to NE in the presence of 1 μM phentolamine (□, ○). Period of NE exposure is indicated by the horizontal bars at the specified concentrations. Symbols represent mean data plus S.E.M.

from control tissue have been reported previously (Garwitz and Jones, 1982). Similar to controls, addition of phentolamine did not alter the basal ^{42}K efflux (0.0182 ± 0.0015) or Δk_{max} (0.0122 ± 0.0006) but shifted the NE EC_{50} to 0.36 ± 0.12 μM ($P < .001$), a dose ratio of 141. The pA_2 ($-\log K_B$) for phentolamine in control animals was 8.13 ± 0.11 ($K_B = 9.1 \pm 2.5$ nM), similar to the phentolamine pA_2 in aorta from aldosterone-treated animals, 8.05 ± 0.11 ($K_B = 10.5 \pm 2.3$ nM).

The effect of increasing phentolamine concentrations on the response of ^{42}K efflux to NE is summarized in figure 2A. Phentolamine produced similar shifts in the parallel dose-response curves of both control and aldosterone groups. The regression line from a Schild plot (not shown) of the data deviates from the required −1.0 (1.11 and 1.27 for aldosterone and control-salt groups, respectively). When the Schild plot is different than 1, the direct linear plot has been recommended as an equivalent way of graphically expressing the same data (Tallarida *et al.*, 1979). The direct linear plot which requires an intercept = 0 and where the K_B is equal to 1/slope (fig. 2B), yields a phentolamine of pA_2 of 8.21 ± 0.01 ($K_B = 6.2 \pm 0.2$ nM), for control aorta and 8.09 ± 0.04 ($K_B = 8.1 \pm 0.8$ nM) for AHR ($P < .05$).

The NE EC_{50} in AHR measured with contraction, 5.3 ± 2.4 nM, agreed closely with the NE EC_{50} obtained by measurement of ^{42}K efflux (2.4 ± 0.5 nM) and was significantly different ($P < .001$) from the contractile EC_{50} for NE, 15 ± 2 nM, in the control-salt group. The effect of phentolamine on the contractile response is presented in figure 3A. The rightward shift in the contractile dose-response curve for both control and AHR tissue was similar to that for ^{42}K efflux. The regression line from the Schild plot of the contractile data for control tissue deviated significantly from −1.0 (0.77) and therefore the data are graphically summarized in a direct linear plot (fig. 3B) which yields a phentolamine pA_2 of 8.13 ± 0.03 ($K_B = 7.4 \pm 0.4$ nM) for control and 8.10 ± 0.05 ($K_B = 7.9 \pm 0.9$ nM) for AHR (P = N.S.).

Prazosin and yohimbine. The effect of the selective *alpha*-1 antagonist prazosin and the *alpha*-2 antagonist yohimbine was evaluated on the catecholamine-stimulated ^{42}K efflux (fig.

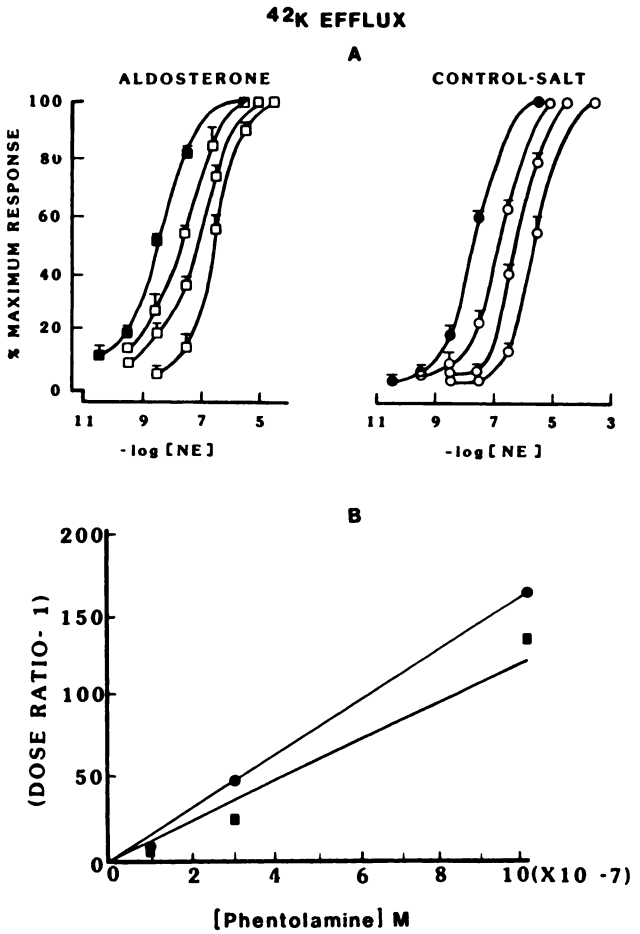

Fig. 2. A, effect of NE on ^{42}K efflux expressed as percentage of maximal response in the absence (closed symbols) and presence (open symbols) of 0.1, 0.3 or 1.0 μM phentolamine in aorta from aldosterone-treated (■, □) and control-salt (●, ○) rats. Symbols represent mean data from six to eight rats for each concentration-response curve. For control tissue, the maximal response (minutes^{-1}) ranged from 0.01092 ± 0.0007 to 0.01312 ± 0.0004 in the absence and 0.01047 ± 0.001 to 0.01252 ± 0.0013 in the presence of phentolamine. For aldosterone-treated tissue, the maximal response ranged from 0.01060 ± 0.0011 to 0.01284 ± 0.0004 in the absence and 0.01166 ± 0.0009 to 0.01207 ± 0.0006 in the presence of phentolamine. B, direct linear plot, constrained to pass through the origin, of the dose ratio − 1 (NE EC_{50} in presence and absence of antagonist) at indicated phentolamine concentrations. K_B = 1/slope.

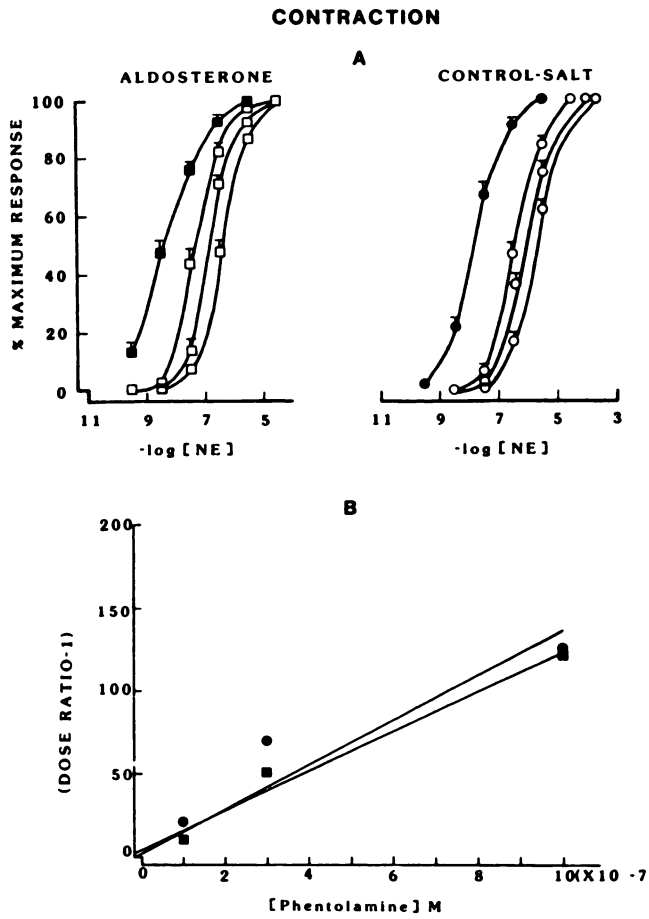

Fig. 3. A, effect of NE on the contractile response, expressed as percentage of maximum response, in the absence (closed symbols) and presence of 0.1, 0.3, or 1.0 μM phentolamine (open symbols) in aorta from aldosterone-treated (■, □; n = 6–7) and control-salt (●, ○; n = 8–9) rats. Values represent mean data for each concentration-response curve. For control tissue, the maximal tension response (grams) ranged from 3.9 ± 0.4 to 4.8 ± 0.2 in absence and 3.0 ± 0.2 to 3.6 ± 0.2 in presence of phentolamine. For aldosterone-treated tissue, the maximal tension response ranged from 3.1 ± 0.5 to 5.0 ± 0.8 in absence and 3.6 ± 0.4 to 5.6 ± 0.6 in presence of phentolamine. B, direct linear plot, constrained to pass through the origin, of the dose ratio − 1 (NE EC_{50} in presence and absence of antagonist) at indicated phentolamine concentrations. K_B = 1/slope.

4) and contraction (fig. 5). It should be noted that a low concentration of prazosin, 0.3 nM, was used to ensure competitive behavior of the *alpha*-1 antagonist as higher prazosin concentrations (>1 nM) produced an apparently noncompetitive decrease in the maximal response and altered the kinetics of the receptor-agonist interaction (data not shown). The pA_2 for the three antagonists (phentolamine, prazosin and yohimbine) was evaluated with both NE and PE used as the agonist and is presented in table 1. The pA_2 for each antagonist was generally similar in the control-salt and AHR, using either ^{42}K efflux or contractile endpoints and NE or PE as agonists. Only one comparison was significantly different (yohimbine, ^{42}K efflux with NE).

Control rats with intact kidneys which drank water (control − H_2O) were evaluated to determine whether nephrectomy and high salt intake altered the tissue response. The pA_2 of the two control groups (water *vs.* salt) was similar for each antagonist with the exception of NE-yohimbine and NE-prazosin (^{42}K efflux). Overall, these results indicate that the nephrectomy and high salt intake procedures did not influence greatly the receptor-agonist interaction.

The antagonist order of potency for either contractile or ^{42}K efflux measurements was prazosin > phentolamine > yohimbine in control-salt, control-water and AHR groups. The normalization of the K_B for yohimbine and prazosin (divided by K_B phentolamine) is presented in table 2. There was no difference between the yohimbine/prazosin ratio in AHR compared to control tissue. The ratios of antagonist potency calculated from radioligand binding data were in good agreement with the ratios from contraction and efflux experiments.

Noncompetitive Antagonist

Angiotensin. The next series of experiments were designed to estimate the K_a for the agonist NE using the method of fractional receptor inactivation by the noncompetitive antagonist Db. Inasmuch as Db is an alkylating agent, initial experiments assessed the specificity of Db inhibition by comparing

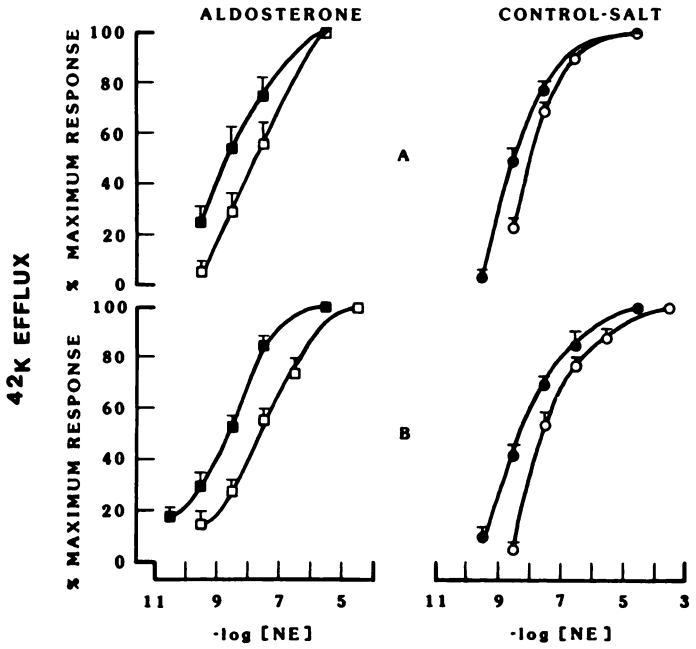

Fig. 4. Effect of NE on ^{42}K efflux, expressed as percentage of maximum response, in absence (closed symbols) and presence (open symbols) of 3×10^{-10} M prazosin (A) or 1.0 μM yohimbine (B) in aorta from aldosterone-treated (●, ○, n = 5–7) and control-salt (●, ○; n = 5–6) rats. Values represent mean data for each concentration-response curve. For control tissue, the maximal response (minutes^{-1}) was 0.0215 ± 0.001 in the absence to 0.0187 ± 0.0011 (prazosin) and 0.0232 ± 0.0024 (yohimbine) in the presence of the antagonists. For aldosterone-treated tissue, the maximal response was 0.112 ± 0.0023 in the absence to 0.0101 ± 0.0015 (prazosin) and 0.0147 ± 0.0011 (yohimbine) in the presence of the antagonists.

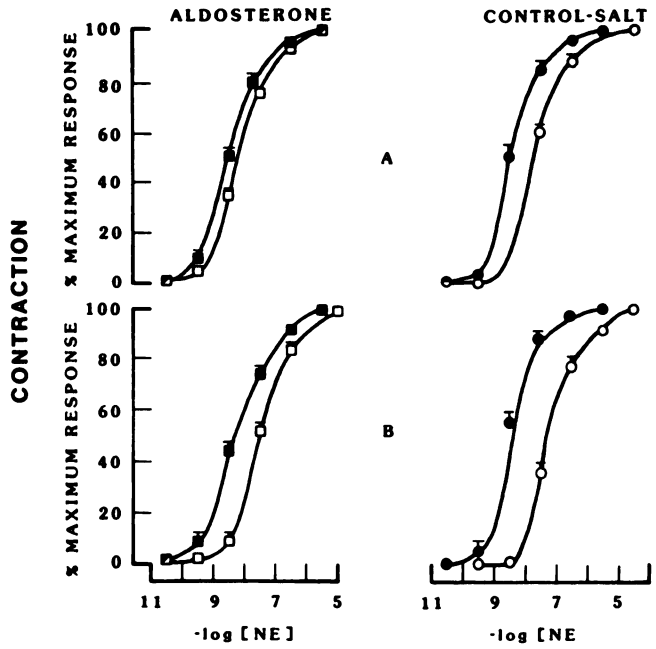

Fig. 5. Effect of NE on the contractile response, expressed as percentage of maximum response, in the absence (closed symbols) and presence (open symbols) of 0.3 nM prazosin (A) or 1.0 μM yohimbine (B) in aorta from aldosterone-treated (■, □; n = 4–6) or control-salt (●, ○; n = 12–13) rats. Values represent mean data for each concentration-response curve. For control tissue, the maximal tension responses (grams) ranged from 3.7 ± 0.3 to 4.5 ± 0.3 in the absence of antagonist and 3.0 ± 0.2 and 3.4 ± 0.3 in the presence of yohimbine and prazosin, respectively. For aldosterone-treated tissue, the maximal tension response ranged from 4.3 ± 0.6 to 4.9 ± 0.8 in the absence of antagonist and 4.9 ± 0.8 in the presence of both yohimbine and prazosin.

the reduction of the angiotensin and NE-induced changes in ^{42}K efflux. Aorta from control and AHR were loaded with ^{42}K and one-half of each aorta was exposed to Db as described above. Each tissue was exposed to a maximal dose of angiotensin, 3 μM, for 5 min, washed for 45 min and then exposed to a maximal dose of NE, 3 μM, for 10 min. The angiotensin-simulated Δk_{max} in control and Db-treated tissues was 0.0145 ± 0.0020 and 0.0132 ± 0.0013, P = N.S. The ratio of the angiotensin Δk_{max}, Db-treated/untreated, 0.96 ± 0.14, (not significantly different from 1) indicated that Db did not inhibit the angiotensin-induced response. In contrast, the NE-induced Δk_{max}, 0.0174 ± 0.0014, was reduced markedly by Db to 0.0049 ± 0.0015, P < .001). The ratio of the NE-induced Δk_{max}, Db-treated/untreated, was 0.28 ± 0.07, significantly different from 1 (P < .001). Similar results were obtained in AHR. Inasmuch as these studies indicated that Db inhibited the NE-induced ^{42}K efflux, Db was then used to estimate the K_a for NE.

Db. The effect of Db on the NE-induced change in ^{42}K efflux in control-salt and AHR is summarized in figure 6A. Treatment with Db did not alter the basal ^{42}K efflux in control (0.0082 ± 0.0002 *vs.* 0.0084 ± 0.0003) or in AHR (0.0170 ± 0.0011 *vs.* 0.0161 ± 0.0012). Db (0.2 μM) decreased the maximum response, Δk_{max}, to 46% of the control response (0.0128 ± 0.0009 to 0.0059 ± 0.0008). However, the AHR required a 2.5 higher concentration of Db, (0.5 μM) to decrease the Δk_{max} to a similar extent (54% of the control response; 0.0123 ± 0.0006 to 0.0066 ± 0.0003). Equieffective NE concentrations before and after Db incubation were determined and the reciprocals of the concentrations were then used to determine the NE K_a for each experiment (see "Methods"). The reciprocals for all the experiments were averaged for each group and the mean data are presented in figure 6B. The NE K_a in AHR, 4.8 ± 1.2 × 10^{-7} M, was the same as the NE K_a in the control group, 4.8 ± 1.5 × 10^{-7} M. Also, the fraction of receptors remaining after Db treatment, or "q", was not significantly different in control and AHR (0.04 ± 0.01 *vs.* 0.02 ± 0.01 respectively, P = N.S.).

The individual K_a values for each experiment were used to calculate the fractional occupancy of receptors by NE. A plot of the relative response for pre-Db concentration response data *vs.* fractional occupancy is presented in figure 7. The graph illustrates that the half-maximal response is obtained with less than 2% of the receptors occupied by NE in AHR whereas the half-maximal response requires occupation of 6% of the receptors by NE in control tissue.

Radioligand binding. Radioligand binding of [^{125}I]HEAT and [^{3}H]prazosin was evaluated in control-salt and hypertensive tissue. The K_D and B_{max} for [^{125}I]HEAT and [^{3}H]prazosin derived from saturation experiments were similar in both control and AHR (table 3). The duration of the hypertensive treatment did not alter these parameters as the 2 and 4 week values were similar. A linear Rosenthal plot of the binding data (not shown) indicated a single class of binding sites in both control and hypertensive aorta.

Inhibition of [^{125}I]HEAT and [^{3}H]prazosin binding by the nonselective agonist NE was also examined (table 4). The Hill coefficient of both groups was less than 1, similar to that reported for control rat aorta (Jones *et al.*, 1987), and therefore calculation of a K_i for NE against [^{125}I]HEAT and [^{3}H]prazosin was not made. However, the NE concentration which inhibited

TABLE 1

Estimate of pA_2 ($-\log K_B$) based on ^{42}K efflux and contraction responses for prazosin (3×10^{-10} M), phentolamine (1×10^{-6} M) and yohimbine (1×10^{-6} M) in aldosterone-treated rats, control-salt rats and rats given tap H_2O to drink

	AHR			Control-Salt			Control-H_2O		
	n	Dose ratio	pA_2	*n*	Dose ratio	pA_2	*n*	Dose ratio	pA_2
					^{42}K efflux				
Prazosin									
NE	7	9 ± 2	10.29 ± 0.13	7	4 ± 1	9.99 ± 0.06†	5	20 ± 6	10.70 ± 0.18
PE	7	58 ± 24	10.63 ± 0.17	5	19 ± 11	10.53 ± 0.21	4	9 ± 2	10.30 ± 0.21
Phentolamine									
NE	7	141 ± 40	8.05 ± 0.11	7	163 ± 40	8.13 ± 0.11	5	144 ± 63	7.96 ± 0.23
PE	7	82 ± 26	7.75 ± 0.17	6	188 ± 50	8.13 ± 0.19	5	137 ± 55	7.97 ± 0.20
Yohimbine									
NE	7	17 ± 4	7.12 ± 0.11*	5	6 ± 1	6.65 ± 0.11†	7	42 ± 16	7.45 ± 0.17
PE	5	20 ± 7	7.14 ± 0.17	6	14 ± 4	7.02 ± 0.12	5	23 ± 7	7.28 ± 0.20
					Contraction				
Prazosin[a]									
NE	5	10 ± 3	10.12 ± 0.14	4	3 ± 1	9.82 ± 0.13	8	5 ± 1	10.09 ± 0.10
PE	6	12 ± 4	10.13 ± 0.15	5	6 ± 2	9.96 ± 0.23	8	14 ± 5	10.42 ± 0.16
Phentolamine									
NE	7	123 ± 44	7.94 ± 0.15	9	127 ± 27	7.92 ± 0.16	6	65 ± 14	7.75 ± 0.10
PE	6	63 ± 27	7.61 ± 0.17	10	30 ± 6	7.35 ± 0.12	6	40 ± 14	7.42 ± 0.18
Yohimbine									
NE	9	8 ± 2	6.74 ± 0.12	7	8 ± 3	6.62 ± 0.18	8	6 ± 1	6.63 ± 0.09
PE	6	35 ± 23	6.99 ± 0.32	5	4 ± 5	6.45 ± 0.07	6	12 ± 5	6.79 ± 0.21

[a] Prazosin, 6×10^{-10} M, was used in the AHR-contractile studies because the dose ratio was minimally shifted by 3×10^{-10} M.

* Significant difference ($P < .05$) between AHR and control-salt using the same agonist; † significant difference ($P < .05$) between control-salt and control-H_2O using the same agonist.

TABLE 2

Relative potencies of *alpha*-adrenergic antagonists

Agonist	Treatment	Contraction			^{42}K Efflux		
		Prazosin/ phentolamine	Yohimbine/ phentolamine	Yohimbine/ prazosin	Prazosin/ phentolamine	Yohimbine/ phentolamine	Yohimbine/ prazosin
NE	Control-H_2O	0.0049	11.1	2260	0.0015	2.6	1730
NE	Control-salt	0.0138	33.4	2420	0.0121	27.4	2260
NE	Aldosterone	0.0059	15.2	2580	0.0067	8.6	1280
PE	Control-H_2O	0.0010	4.7	4700	0.0038	4.9	1290
PE	Control-salt	0.0026	6.0	2310	0.0030	8.7	2900
PE	Aldosterone	0.0030	7.7	2570	0.0010	3.3	3330
	Treatment	$[^3H]$Prazosin Binding[a]			$[^{125}I]$HEAT Binding[a]		
	Control-salt	0.013	31	2380	0.005	15.7	3140

[a] Radioligand binding data from Jones *et al.* (1987).

50% of the antagonist binding (IC_{50}) is similar in the control and AHR for either antagonist. In order to test the hypothesis that the increased sensitivity of the AHR tissue to NE was the result of an increased density of *alpha*-2 adrenergic receptors, saturation studies of *alpha*-2 receptors were conducted. The K_D (0.74 and 0.59 nM) and B_{max} (9.6 and 9.8 fmol/mg) for $[^3H]$ yohimbine was similar in control-salt and hypertensive tissue, indicating no increase in *alpha*-2 receptor density.

Discussion

Pharmacologic evaluation of the *alpha* adrenergic receptor using both contraction and ^{42}K efflux indicates that the receptor affinity as well as the subtype (*alpha*-1) is not altered in the aorta from AHRs. In addition, radioligand binding studies indicate that the receptor affinity and density are similar in control and AHR tissue. Therefore, it can be concluded that alterations in the *alpha* adrenergic receptor or in the receptor-agonist interaction do not underlie the catecholamine supersensitivity which is characteristic of aortic smooth muscle from AHR and that postreceptor events mediate this phenomenon.

Initial experiments for the nonselective competitive antagonist phentolamine determined that the pA_2 was similar in aorta from hypertensive and control-salt rats (table 1). These pA_2 values, obtained from both contractile and ^{42}K efflux measurements, were comparable to those reported previously for control rat aorta ($pA_2 = 8.1$; Digges and Summers, 1983; Decker *et al.*, 1984; Hamed *et al.*, 1983). It is not clear why the regression line of the Schild plot for phentolamine was different from the predicted 1 for control ^{42}K efflux (1.27) as well as contractile measurements (0.77), but similar findings have been reported by several investigators (Digges and Summers, 1983; Randriantsoa *et al.*, 1981; Hamed *et al.*, 1983). Nerves in the rat aorta (Burnstock, 1975), an uptake-2 mechanism (Koehler *et*

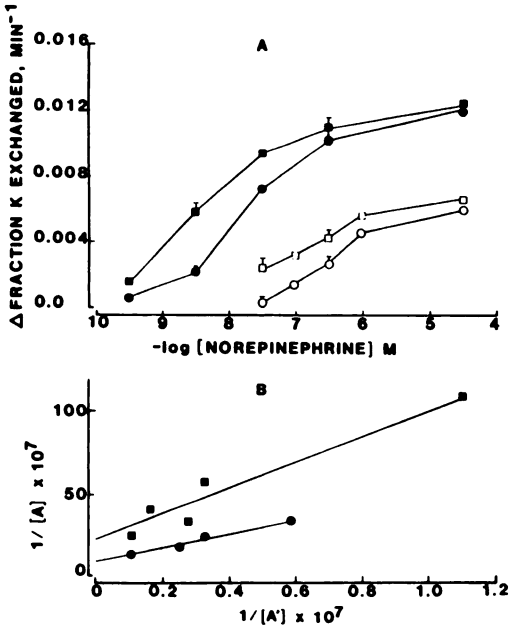

Fig. 6. A, effect of NE on ^{42}K efflux in aorta from aldosterone-treated (squares; $n = 9$) and control-salt (circles; $n = 7$) rats in the presence and absence of Db. One-half of each aorta was exposed to NE under control conditions (closed symbols) whereas the other half was exposed to Db (open symbols; aldosterone, 5×10^{-7} M Db and control, 2×10^{-7} M) for 15 min. Symbols represent mean data ± S.E.M. B, double reciprocal plot of equieffective NE concentrations before and after Db treatment (data obtained from A). Lines were derived from linear regression methods.

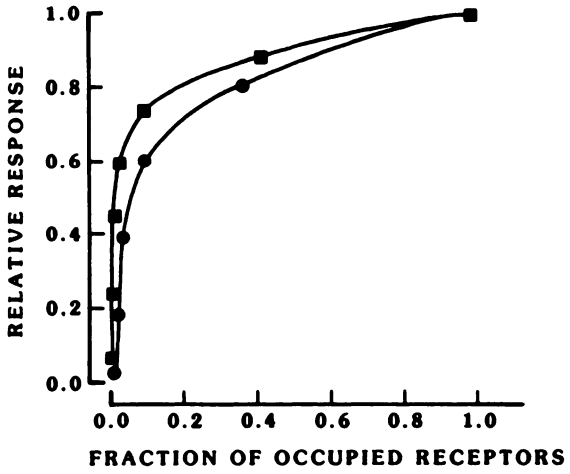

Fig. 7. Effect of NE on ^{42}K efflux (presented as percentage of maximum response) as a function of the fraction of receptors occupied by NE ($RA/RT = [NE]/K_a + [NE]$ in which RA is receptor-agonist complex and RT is the total number of receptors) in aldosterone-treated (■, $n = 9$) and control-salt (●, $n = 7$) rats. NE K_a derived from each experiment used to calculate fraction of occupied receptors. Response data same as that presented in figure 6A for untreated tissues (solid symbols). Symbols represent mean data ± S.E.M.

al., 1979), and insufficient equilibration time (Kenakin, 1980; pilot experiments) were ruled out as contributing factors.

Measurement of ^{42}K efflux or contraction in the presence of the selective antagonists prazosin (*alpha*-1) and yohimbine (*alpha*-2) also indicated there were no changes in the hypertensive tissue. The pA_2 values for prazosin obtained with ^{42}K efflux were the same (table 1) in AHR and control-salt tissues. These values agree with the higher affinity of prazosin that has been

TABLE 3

[^{125}I]HEAT and [^{3}H]prazosin binding to *alpha*-1 adrenergic receptors in aorta from control-salt and AHRs

	Control		n	Hypertensive	
	K_D	B_{max}		K_D	B_{max}
	pM	fmol/mg		pM	fmol/mg
[^{125}I]HEAT					
2 weeks	19 ± 2	38 ± 6	4	29 ± 8	39 ± 4
4 weeks	18 ± 1	29 ± 1	4	25 ± 2	28 ± 4
[^{3}H]Prazosin					
2 weeks	34 ± 6	44 ± 3	4	26 ± 3	39 ± 1
4 weeks	30 ± 2	44 ± 6	3	35 ± 2	42 ± 4

TABLE 4

Inhibition of [^{125}I]HEAT and [^{3}H]prazosin binding by NE in aorta from AHR and control rats

	[^{125}I]HEAT IC_{50}	n	[^{3}H]Prazosin IC_{50}	n
	nM		nM	
Control-salt	175 ± 32	3	188 ± 23	2
Hypertensive	109 ± 24	3	118 ± 11	3

reported for rat aorta (Agrawal *et al.*, 1984). The yohimbine pA_2 assessed by ^{42}K efflux and PE was the same in control-salt and AHR (table 1). Estimation of antagonist pA_2 using contractile measures also indicated that these values for prazosin or yohimbine were similar in AHR and control-salt groups. The close correlation between the receptor-mediated processes of ^{42}K efflux and contraction observed in control tissues (Smith and Jones, 1985) is reflected by the comparable values for antagonist pA_2 obtained by measurement of ^{42}K efflux and contraction. The pA_2 values for yohimbine were more variable but this may reflect the high (micromolar) concentrations of the *alpha*-2 antagonist required to antagonize the response.

The rank order of potency for antagonist inhibition of both ^{42}K efflux and contraction is prazosin > phentolamine > yohimbine in AHR and both control groups. Radioligand binding studies (Jones *et al.*, 1987) give the same rank order for antagonists in control rat aorta using both [^{125}I]HEAT and [^{3}H] prazosin as radioligands. These results along with the yohimbine-prazosin potency ratio (table 2) indicate that the predominate receptor type in rat aorta is *alpha*-1 which is not changed in the hypertensive animal. It should be pointed out that the prazosin pA_2 obtained with the nonselective agonist NE and the selective agonist PE are identical which is compatible with an *alpha*-1 subtype. These findings agree with recent reports (Decker *et al.*, 1984; Digges and Summer, 1983; Hamed *et al.*, 1983; Macia *et al.*, 1984) which have classified the primary receptor type in control rat aorta as the *alpha*-1 subtype. Also, the underlying assumption that the antagonists were acting in a competitive fashion is supported by the similar pA_2 values derived for each antagonist using either agonist.

The *alpha*-1 adrenergic receptor was also evaluated with the radioligands [^{3}H]prazosin and [^{125}I]HEAT and the results from these studies confirm those obtained with the competitive antagonists. The prazosin K_D, consistent with values reported previously (0.05–0.78 nM, Bylund and U'Prichard, 1983) was similar in the control-salt and AHR membranes (table 3), which indicates that receptor affinity for antagonists was not increased in the hypertensive tissue. It should be pointed out that the [^{3}H]prazosin K_D agrees with the prazosin K_B obtained by ^{42}K efflux or contractile measurements which suggests that these procedures are evaluating the same receptor. The radio-

ligand studies also indicated that an increase in receptor density was not responsible for the NE supersensitivity in AHR inasmuch as the B_{max} for [^{125}I]HEAT and [^{3}H]prazosin was similar in aorta from control-salt and AHR (table 3). These findings are in contrast to reports that the density of *alpha*-1 adrenergic receptors is elevated in various tissues from hypertensive rats (Gheyouche *et al.*, 1980; Kobayashi *et al.*, 1985; U'Prichard *et al.*, 1979; Yamada *et al.*, 1980). However, these increases occurred in cerebral microvessels and cerebral tissues whereas the evaluation of peripheral vascular tissue has not been studied extensively.

Agonist receptor affinity can also be evaluated by the measurement of the dissociation constant (K_a) for NE in functional studies and the NE K_D in radioligand binding studies. The K_a values were identical in control and hypertensive tissues (fig. 6) (4.8×10^{-7} M) and agree with the NE K_a values reported by other investigators for rat, 4.3×10^{-7} M (Digges and Summers, 1983) and rabbit, 3.4×10^{-7} M (Besse and Furchgott, 1976). Similar findings were reported in the spontaneously hypertensive rat (Strecker *et al.*, 1975) in which the NE K_a in normotensive rats, 1.8×10^{-7} M and hypertensive rats, 1.3×10^{-7} M, was not significantly different. The NE K_D could not be calculated accurately which limits the conclusion that can be drawn from the radioligand binding studies. However, the NE IC_{50} values for inhibition of [^{3}H]prazosin (1.8×10^{-7} M) and [^{125}I]HEAT (1.9×10^{-7} M) were consistent with the NE K_a.

The NE EC_{50} values for activation of ^{42}K efflux and contraction in control-salt group (3.5×10^{-8} M and 1.5×10^{-8} M, respectively) are 5- to 32-fold less than the NE K_a obtained from fractional receptor inactivation (4.8×10^{-7} M) or the IC_{50} (1.8×10^{-7} M) for the NE inhibition of [^{3}H]prazosin and [^{125}I] HEAT binding. This nonlinear relationship between receptor occupancy and the functional response has been used to suggest that there are spare receptors in various tissues including rat aorta (Digges and Summers, 1983). Although a nonlinear relation was observed in our study (fig. 7), almost 100% occupancy was required for a 100% response. However, because only one concentration of Db was used in this study, insufficient information is available to draw conclusions concerning the presence or absence of spare receptors.

It is apparent from figure 7 that the responsiveness to NE in AHR is enhanced because, at the half-maximal response, only 2% of the receptors are occupied in contrast to 6% occupancy

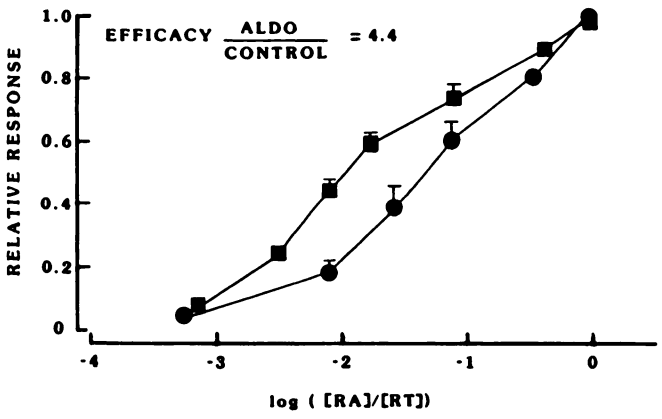

Fig. 8. Comparison of the efficacy of NE in aldosterone (ALDO)-treated (■) and control-salt (●) rats. Replot of the data in figure 7 using the log of the fraction of occupied receptors. The efficacy of NE in ALDO-treated relative to control rats is given by the antilog of the distance between the two curves.

in the controls, a value identical to that reported for rabbit (Besse and Furchgott, 1976). Inasmuch as uptake mechanisms, receptor affinity and receptor number have not changed in hypertensive tissue, it appears that increased efficacy, reflecting receptor coupling to internal biochemical events, underlies the enhanced adrenergic responsiveness in the hypertensive tissue. It can be seen readily in figure 8 that at an equivalent receptor occupation (log = −2.1), the relative response in the AHR tissues is 42 ± 3% whereas, in the control tissue, the relative response is 18 ± 3% ($P < .001$). The efficacy of the response in AHR relative to that of control, which is the antilog of the distance between the two curves (Furchgott, 1966; Besse and Furchgott, 1976), is 4.4. This increased efficacy in hypertensive tissue may explain why the hypertensive tissue required 2.4-fold more Db than controls to achieve a similar reduction in the maximal response (fig. 6), despite equivalent receptor density in the two tissues (table 3).

We conclude from this study that there is no alteration in the *alpha* adrenergic receptor affinity, number or subtype in the AHR aorta and that postreceptor events underlie the phenomenon of catecholamine supersensitivity in AHR. These results are consistent with the nonspecific nature of the supersensitivity which extends to a variety of nonadrenergic agonists in addition to NE (Jones *et al.*, 1981). One possible link between these varied agonists is that they bind to receptor systems coupled to calcium mobilization (Gill, 1985). Because calcium metabolism is altered in hypertensive tissue (Kwan, 1985), it is possible that the mechanisms which translate receptor occupation into an elevation of cytoplasmic calcium may underlie the increased agonist efficacy. One candidate is the increased production of second messengers by the phosphatidylinositol pathway which generate intracellular signals for calcium mobilization (Berridge and Irvine, 1984). Extensive study will be required to delineate the specific mechanism underlying the increased efficacy in the hypertensive tissue.

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