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Interactions of Amiloride with α- and β-Adrenergic Receptors: Amiloride Reveals an Allosteric Site on α_2-Adrenergic Receptors

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SUMMARY

Interaction of amiloride with adrenergic receptors was studied using radioligand binding techniques. Amiloride competed for [^{3}H]prazosin binding to α_1-adrenergic receptors on rat renal cortical membranes and BC3H-1 muscle cell membranes. Nonlinear regression analysis of radioligand binding isotherms showed that amiloride increased K_d without a change in B_{max}, suggesting the drug binds competitively in a mutually exclusive manner with the radioligand at the receptor-binding site. Similarly, amiloride competitively blocked [^{125}I]iodocyanopindolol binding to β-adrenergic receptors on both tissues. The addition of guanylyl 5′-imidodiphosphate or sodium chloride did not alter the interaction of amiloride with α_1- or β-adrenergic receptors. The interaction of amiloride with α_2-adrenergic receptors was more complex and revealed an allosteric site. In both rat renal cortical membranes and intact human platelets, amiloride increased the K_d for [^{3}H]rauwolscine binding, as well as decreasing the apparent B_{max}. In binding experiments where amiloride competed for [^{3}H]rauwolscine-binding sites, pseudo-Hill slopes of less than 1.0 were obtained for both platelet and renal α_2 receptors. In addition, amiloride increased the rate of [^{3}H]rauwolscine dissociation from renal α_2 receptors. In the presence of 100–120 mM sodium chloride, the K_i for amiloride competition was decreased an average of 54% in renal membranes; in contrast, sodium increased the K_d of the agonist epinephrine. Taken together, these data support the hypothesis that α_2-adrenergic receptors, but not α_1- or β-adrenergic receptors, have an allosteric site to which amiloride binds and which we propose to be a cation-binding site.

The pyrazine derivative, amiloride, has been used in a variety of tissues to probe membrane-associated macromolecules having sodium-binding sites, including Na^+/H^+ and Na^+/Ca^{2+} exchangers and certain types of sodium channels (1–3). We recently reported that α_2-adrenergic agonists stimulate sodium flux in renal proximal tubular cells and that this response could be inhibited by amiloride at concentrations (μM to mM) that are known to block the Na^+/H^+ exchanger (4, 5). Subsequent studies have shown that amiloride and certain of its analogues are able to inhibit radioligand binding to α_2-adrenergic receptors as well as α_1- and β-adrenergic receptors in rat renal cortical membranes (6). Additionally, amiloride blocked α_1-adrenergic receptor-mediated functional response in kidney cells in tissue culture (6). In the current study, we performed a more detailed characterization of the interaction of amiloride at α_1-, α_2-, and β-adrenergic receptors from several tissues. We show that the interaction of amiloride with these receptors is different depending on the receptor subtype, that the interaction is not tissue specific, and that this interaction is modulated by sodium at α_2-adrenergic receptors. The data suggest that amiloride is a receptor antagonist for all types of adrenergic receptors. At α_2-adrenergic receptors, amiloride may also bind to an allosteric site in addition to the receptor-binding site.

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Experimental Procedures

Materials. Tissue culture dishes were obtained from Costar. Serum and minimal essential medium were from Gibco Laboratories (Grand Island, NY). [^{3}H]Prazosin (82 Ci/mmol) and [^{3}H]rauwolscine (88.7 Ci/mmol) were purchased from New England Nuclear Corporation. (−)-Cyanopindolol was a gift from G. Engel (Sandoz). Iodine-125 was purchased from New England Nuclear; cyanopindolol was iodinated according to the method of Engel *et al.* (7). Amiloride was purchased from Sigma and dissolved for each experiment in ethanol/dimethyl sulfoxide, 1:1; this solvent was without effect in control experiments at the concentrations used in the incubations.

Tissue preparation. Membranes were prepared from rat renal cortex (8), BC3H-1 muscle cells (9), and platelets (10) as previously described. Intact platelets were obtained from fresh blood as previously described (10).

Radioligand binding assays. α_1- and α_2-Adrenergic receptors were identified in radioligand binding assays by the use of the specific antagonists [^{3}H]prazosin and [^{3}H]rauwolscine, respectively (6, 8–10). Nonspecific binding was defined in the presence of 10 μM phentolamine for both α subtypes of adrenergic receptor. (−)-[^{125}I]ICYP was used to characterize β-adrenergic receptors using 1.0 μM propranolol to define nonspecific binding (9, 11).

Renal cortical membranes. For all studies, membranes were

ABBREVIATIONS: ICYP, iodocyanopindolol; EDTA, ethylenediaminetetraacetic acid; Gpp(NH)p, guanylyl 5′-imidodiphosphate.

added to tubes containing radioligand and competing agent, and samples were incubated at 25° for 1 hr in the case of α-adrenergic receptors and 1.5 hr in the case of β-adrenergic receptors. The total volume of the assays varied from 0.25 to 1.0 ml in different experiments. The incubation buffer consisted of 50 mM Tris-HCl, 10 mM $MgCl_2$ ($MgCl_2$ was not included for the α_2 receptor binding studies to obviate reduction of receptor affinity), and 0.5 mM EDTA, pH 7.5. Assays were terminated by the addition of 10 ml of incubation buffer (ice-cold for α_2 receptor studies in order to reduce dissociation of the ligand), and the bound and free radioligand were separated by filtration through Whatman GF/C glass-fiber filters on a Brandell Cell Harvestor (#N-48R). The radioactivity trapped on the filters was quantitated by liquid scintillation spectrometry at 40% counting efficiency for tritiated ligands and by gamma counting for iodinated ligands at 85% efficiency. Protein for these and other tissues was determined by the method of Lowry *et al.* (12), using bovine serum albumin as standard.

BC3H-1 membranes. [^{3}H]Prazosin binding was assayed in a final volume of 0.5 ml of buffer (50 mM Tris-HCl, 10 mM $MgCl_2$, pH 7.6) at a protein concentration of 0.1–0.3 mg/ml as described previously (9). The binding reactions were carried out for 60 min at 30° in a shaking water bath. To terminate the binding reaction, 10 ml of buffer (5.0 mM Tris-HCl, pH 7.4) were added to each tube and the membranes were filtered on Whatman GF/C glass-fiber filters and washed with a further 10 ml of buffer. [^{125}I]Iodocyanopindolol (ICYP) was used to assess β-adrenergic receptor interactions by the same procedure.

Platelets. [^{3}H]Rauwolscine binding assays were carried out in a final volume of 0.25 ml of incubation buffer in a shaking water bath at 25° for 45 min as previously described (10). The reactions were terminated by the addition of 10 ml of PBS and filtration over Whatman GF/C glass-fiber filters followed by a wash with a further 10 ml of PBS.

Determination of ligand dissociation rate. For the determination of radioligand dissociation rate, renal cortical membranes were equilibrated with 5.0 nM [^{3}H]rauwolscine for 90 min. Dissociation was initiated by diluting 1:40 with incubation buffer in the presence or absence of 100 μM or 300 μM amiloride. Bound and free radioligand were separated and quantitated as above.

Data analysis. Competitive binding curves were analyzed by computer using iterative, nonlinear regression to determine K_i values and pseudo-Hill slopes (13).

Saturation binding isotherms were analyzed by iterative nonlinear regression (Marquardt) to determine the maximum number of binding sites (B_{max}) and dissociation constants (K_d). These data are displayed as Scatchard plots. The lines in these plots were determined by the K_d and B_{max} values obtained by nonlinear regression. Inhibitor constant (K_i) values were calculated according to the method of Cheng and Prussoff (14).

To compare K_d values, we used a one-sample, one-tailed t test to determine whether the ratio of treated to control values was greater than 1.0. B_{max} and K_i values were compared by paired one-tailed Student's t test; values are presented as mean ± standard error unless otherwise stated. K_i values were calculated, in several cases, from analysis of saturation binding isotherms according to the following equation: $K_i = [\text{amiloride}]/((K_d^*/K_d) - 1)$, where K_d^* = apparent K_d of radioligand in the presence of amiloride. Each figure is a single representative experiment; each experiment was repeated at least three times unless otherwise stated.

Results

We have shown previously that amiloride inhibits radioligand binding to adrenergic receptors in rat renal cortical membranes with a rank order of potency $\alpha_2 > \alpha_1 > \beta$ (6). We sought initially to determine the type of interaction that was responsible for the inhibition of radioligand binding.

Amiloride competitively inhibits radioligand binding at α_1- and β-adrenergic receptors. For renal cortical membranes we evaluated the binding of [^{3}H]prazosin to α_1-adrenergic receptors and found that amiloride increased the apparent dissociation constant (K_d) of radioligand in a dose-dependent manner (Fig. 1A). The K_d of [^{3}H]prazosin increased from control levels of 0.14 ± 0.02 nM to 0.62 ± 0.21 nM in the presence of 100 μM amiloride ($N = 4$, $p < 0.05$) and 1.7 ± 0.7 nM in the presence of 300 μM amiloride ($N = 4$, $p < 0.05$). The maximal number of binding sites (B_{max}) was not changed (control = 72 ± 6 fmol/mg of protein; 80 ± 12 fmol/mg of protein in the presence of 100 μM amiloride; 69 ± 5 fmol/mg of protein in the presence of 300 μM amiloride), a result expected for competitive blockade. The calculated K_i value of 34 ± 7 μM for amiloride is consistent with the directly measured value of 24 ± 7 μM (6).

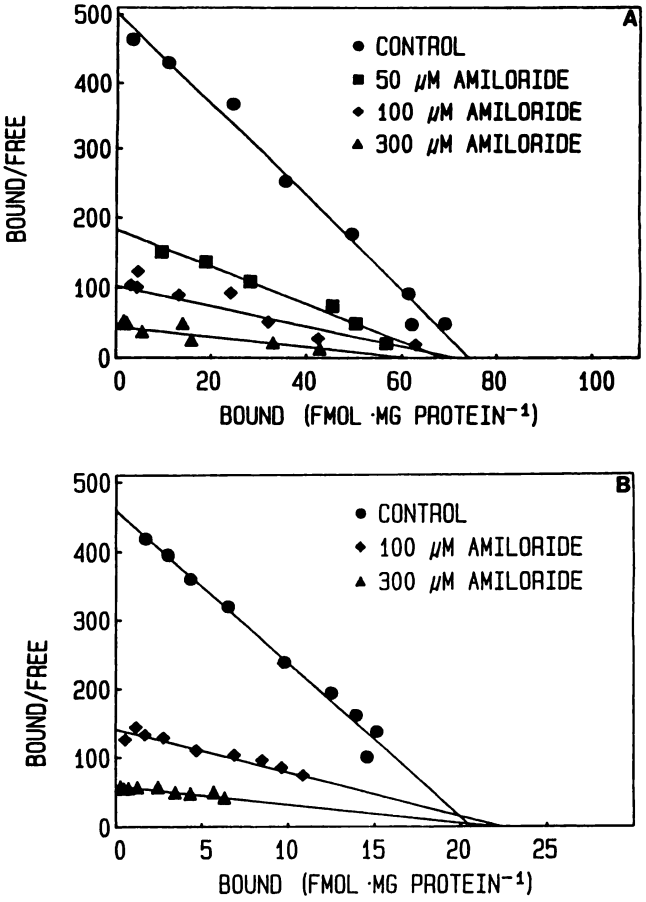

Fig. 1. Competition of amiloride for radioligand binding to α_1- and β-adrenergic receptors on renal cortical membranes. A. Analysis of [^{3}H]prazosin binding in the presence or absence of fixed concentrations of amiloride. Renal membranes were incubated with increasing concentrations of [^{3}H]prazosin for 1 hr at 25°. B. Scatchard analysis of [^{125}I]ICYP binding in the presence or absence of a fixed concentration of amiloride. Renal cortical membranes were incubated with increasing concentrations of radioligand for 90 min at 25°.

In order to determine whether the interaction of amiloride with renal α_1 receptors was reversible, we attempted to reverse the effect of amiloride by washing the treated membranes. The K_d for [^{3}H]prazosin increased from 0.19 ± 0.08 nM to 0.48 ± 0.07 nM (mean ± SD) in the presence of 100 μM amiloride ($N = 2$, $p < 0.07$ by one-sample, one-tailed t test). After control and amiloride-treated membranes were washed three times, the K_d values for [^{3}H]prazosin were identical (0.26 nM). Therefore,

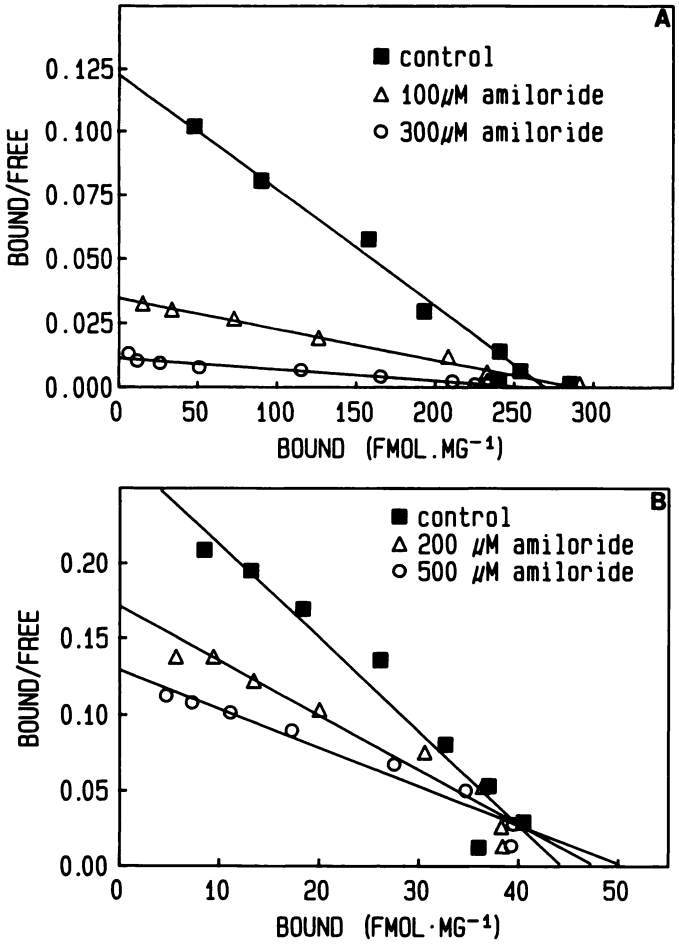

Fig. 2. Competitive interactions of amiloride for radioligand binding to BC3H-1 cell membranes. A. Saturation isotherms of [^{3}H]prazosin binding to BC3H-1 cell membranes in the presence and absence of amiloride. BC3H-1 cell membranes were incubated at 30° for 1 hr. B. [^{125}I]ICYP binding to BC3H-1 cell membranes in the presence and absence of amiloride. Incubation conditions were as in A.

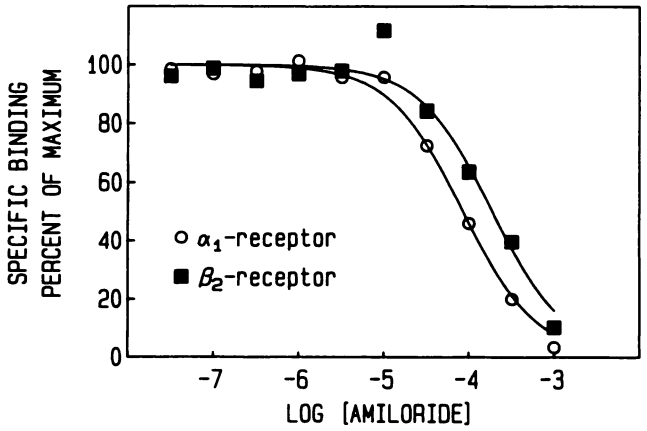

Fig. 3. Competition of amiloride for [^{3}H]prazosin or [^{125}I]ICYP-binding sites on BC3H-1 cell membranes. BC3H-1 cell membranes were incubated with increasing concentrations of amiloride in the presence of 100 pM [^{3}H]prazosin (○) or 100 pM [^{125}I]ICYP (■). Specific binding was determined as described in Experimental Procedures.

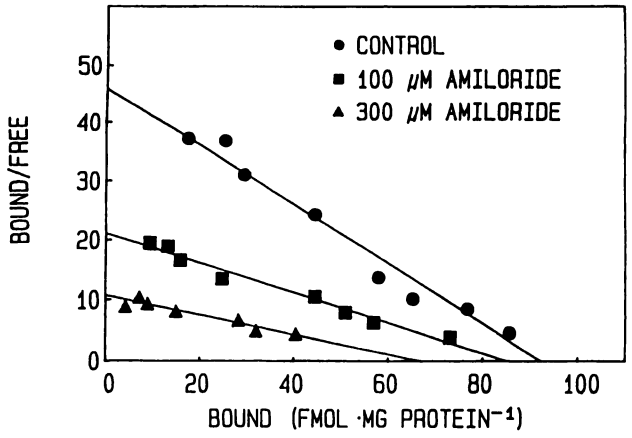

Fig. 4. Amiloride interacts with renal α_2-adrenergic receptors in a complex manner. Renal cortical membranes were incubated with increasing concentrations of [^{3}H]rauwolscine in the presence or absence of a fixed concentration of amiloride for 1 hr at 25°.

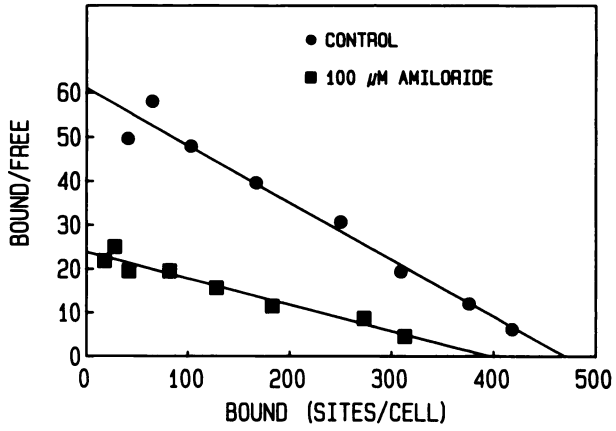

Fig. 5. Effect of amiloride on [^{3}H]rauwolscine binding to human platelets. Freshly isolated platelets were incubated with increasing concentrations of radioligand with or without 100 μM amiloride.

the interaction of amiloride at renal α_1-adrenergic receptors was completely reversible.

The interaction of amiloride at renal β-adrenergic receptors was also competitive, showing an increase in the apparent K_d for [^{125}I]ICYP binding, with no change in B_{max} (Fig. 1B). The K_d was significantly increased ($N = 5$, $p < 0.01$) from control values of 44 ± 1.2 pM to 105 ± 18 pM in the presence of 100 μM amiloride and 227 ± 53 pM in the presence of 300 μM amiloride, but the maximal number of binding sites was unchanged (19.6 ± 0.8, 23 ± 2, and 23.7 ± 1.6 fmol/mg of protein, respectively). The calculated K_i for amiloride of 95.4 ± 15.2 μM is comparable to the measured K_i of 83.6 ± 13.5 μM (6) for renal β-adrenergic receptors.

To determine whether the interaction of amiloride with these renal receptors was tissue specific, similar experiments were conducted on membranes from BC3H-1 cells; these muscle cells have both α_1- and β_2-adrenergic receptors (9). In the BC3H-1 cell membranes, amiloride competitively blocked radioligand binding at α_1-adrenergic receptors (Fig. 2A). The apparent K_d of [^{3}H]prazosin was increased from a mean control value of 0.15 nM to 0.42 nM ($N = 2$) in the presence of 100 μM amiloride and 1.1 nM ($N = 2$) in the presence of 300 μM amiloride (Fig. 2A). The K_i for amiloride (33 μM, Fig. 3) at BC3H-1 α_1 receptors was virtually identical to that found at renal α_1-adrenergic receptors (34 ± 7 μM).

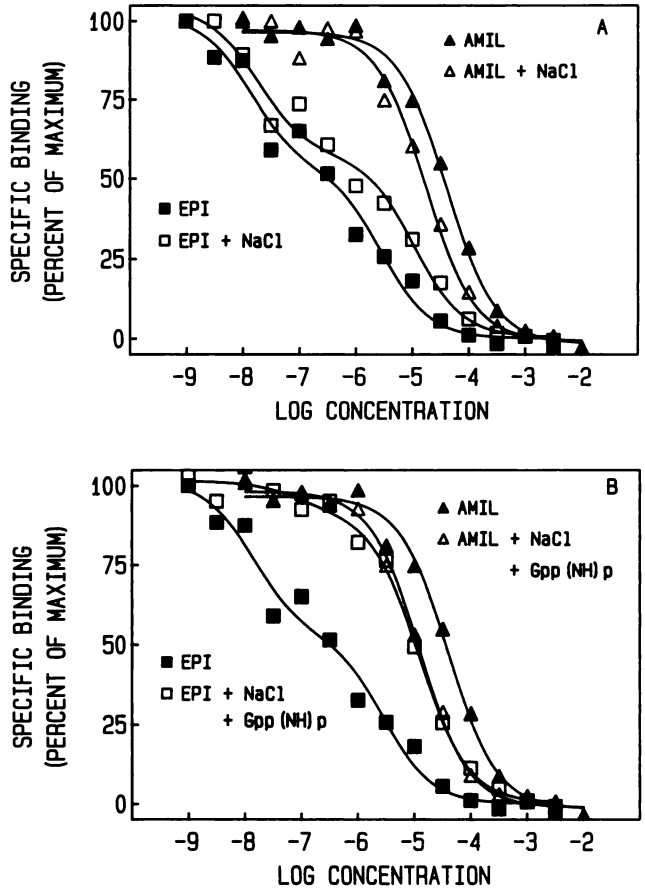

Fig. 6. Effect of Gpp(NH)p and sodium chloride on competition of epinephrine (*EPI*) and amiloride (*AMIL*) for [^{3}H]rauwolscine-binding sites in rat renal cortical membranes. A. Renal cortical membranes were incubated with increasing concentrations of epinephrine or amiloride, with and without 120 mM NaCl, and 4.7 nM [^{3}H]rauwolscine. B. Renal cortical membranes were incubated with increasing concentrations of epinephrine or amiloride with and without 120 mM NaCl and 100 μM Gpp(NH)p.

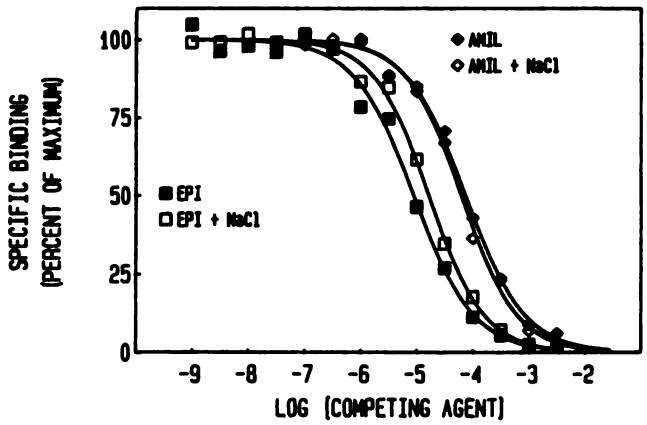

Fig. 7. Effect of sodium chloride on competition of epinephrine (*EPI*) and amiloride (*AMIL*) for [^{3}H]prazosin-binding sites in rat renal cortical membranes. Renal cortical membranes were incubated with increasing concentrations of epinephrine or amiloride with and without 120 mM NaCl. [^{3}H]Prazosin (0.2 nM) was used as radioligand.

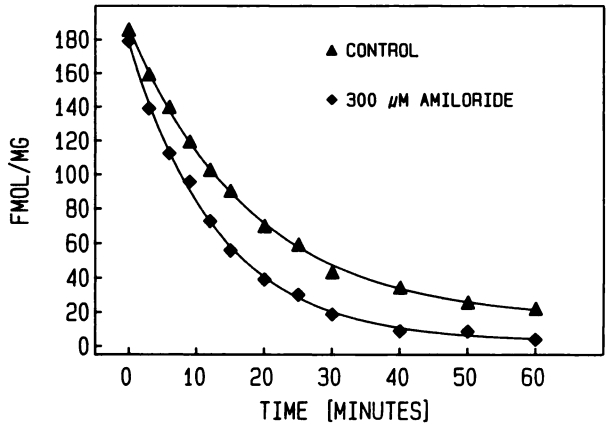

Fig. 8. Effect of amiloride on dissociation of [^{3}H]rauwolscine from rat renal cortical membranes. Renal cortical membranes were preincubated with [^{3}H]rauwolscine for 90 min and then dissociation was initiated by dilution of the membranes into buffer in the presence or absence of 300 μM amiloride. Data shown are similar to those obtained in five experiments and were fit by computer to a single exponential (13). The $t_{½}$ for the control was 12.42 min and for 300 μM amiloride it was 9.05 min. The dissociation plateaued above 0 as predicted for dilution of ligand to a final concentration of 0.125 nM.

Analogous to results observed for the renal β receptor, the presence of amiloride increased the mean K_d for [^{125}I]ICYP binding to β-adrenergic receptors in BC3H-1 cell membranes from 23 pM to 47 pM (N = 2) in the presence of 200 μM amiloride and 85 pM (N = 2) in the presence of 500 μM amiloride (Fig. 2B); B_{max} was not affected by amiloride. The K_i for amiloride at BC3H-1 membrane β_2 receptors, determined in competition binding studies (Fig. 3), was 60 μM compared to 83 ± 14 μM in kidney membranes, indicating similarity in the interaction even though kidney membranes possess 70% β_1 receptors (11) and BC3H-1 cells have only β_2-adrenergic receptors (9).

Amiloride has a mixed type of inhibition at α_2-adrenergic receptors. Unlike the classic competitive interaction between amiloride and radioligand at α_1- and β-adrenergic receptors, amiloride had a more complicated interaction at α_2-adrenergic receptors. In studies with renal cortical membranes, we found an increase in apparent K_d of [^{3}H]rauwolscine from control values of 2.4 ± 0.3 nM (N = 4) to 4.8 ± 0.8 nM in the presence of 100 μM amiloride (N = 4, $p < 0.01$) and 9.0 ± 2.5 nM (N = 3, $p < 0.05$) in the presence of 300 μM amiloride (Fig. 4). In addition, the maximal number of binding sites was significantly reduced ($p < 0.01$) from 83.2 ± 6 to 66 ± 10 fmol/mg of protein in the presence of 100 μM amiloride and from 97 ± 5 to 72 ± 6 fmol/mg of protein (N = 3, $p < 0.05$) in the presence of 300 μM amiloride.

In order to determine whether this mixed type of interaction was found for α_2-adrenergic receptors in other tissues, we conducted similar experiments using human platelets (Fig. 5). In platelets, the apparent K_d for [^{3}H]rauwolscine increased from 6.6 ± 0.7 to 12.7 ± 3.7 nM (N = 3, $p < 0.06$) in the presence of 100 μM amiloride. The number of apparent binding sites/cell decreased by 17 ± 2.6% ($p < 0.01$).

In order to be sure that the mixed type of inhibition at α_2-adrenergic receptors was not an artifact of the experimental procedure, we tested the reversibility of the interaction of renal α_2 receptors and found that the effects of amiloride on K_d and B_{max} ($p < 0.05$) were completely reversed (N = 3) when the

membranes were washed (data not shown). This result indicates that amiloride was unlikely to have nonspecific or disruptive effects on membranes or α_2 receptors.

Interaction of amiloride and sodium with α-adrenergic receptors. Agonist versus antagonist binding at adrenergic receptors can be distinguished by effects of added guanine nucleotides and/or sodium. The nonhydrolyzable analogue of GTP, Gpp(NH)p, reduces receptor affinity for agonist at adrenergic receptors in renal membranes while having no effect on antagonist binding (8, 11, 15). As illustrated in Fig. 6B, Gpp(NH)p lowers the affinity for epinephrine and converts a mixed population of high and low affinity receptor-binding sites to a single low affinity population of renal α_2-adrenergic receptors. In contrast, Gpp(NH)p had no effect on competition of amiloride for [^{3}H]rauwolscine-binding sites, either alone (data not shown) or in the presence of 120 mM NaCl (cf. Fig. 6, A and B).

Sodium chloride, which also lowers renal α_2 receptor affinity for epinephrine (8, 15), had no effect on the affinity "state" of the receptor as evidenced by the parallel shift to the right in the competitive binding curves (Fig. 6A). Unlike the results with epinephrine, in the presence of 100–120 mM NaCl, the apparent affinity of renal α_2 receptors for amiloride was increased, as indicated by a *left* shift in the competitive binding curve (Fig. 6A). In a series of paired experiments, the K_i of amiloride was decreased from control values of 20.1 ± 3.0 μM to 6.3 ± 0.8 μM ($N = 6$, $p < 0.01$) in the presence of 100–120 mM sodium chloride. The pseudo-Hill slope was 0.83 ± 0.05 in the control and 0.9 ± 0.04 in the presence of sodium. The possible effects of ionic strength were tested by substituting *N*-methyl-D-glucamine chloride for sodium; the affinity for amiloride was not different from control under these circumstances ($N = 3$, data not shown). In order to determine whether the effect of sodium was specific for this cation, lithium chloride (0–250 mM) was tested for its ability to shift the K_i for amiloride in renal membranes and was without effect (data not shown).

The effect of sodium on the interaction of amiloride with α_2 receptors was also examined in studies with platelet membranes. In two experiments, the apparent K_i was reduced 7- and 3-fold by 100 mM NaCl. In four other experiments, the K_i was not changed or decreased less than 2-fold. Overall, the K_i was 8.0 ± 2.1 μM and the pseudo-Hill slope was 0.67 ± 0.09 in control membranes ($N = 6$, *N*-methyl-D-glucamine chloride added to control for ionic strength effects) and was 8.0 ± 0.7 μM with a pseudo-Hill slope of 0.66 ± 0.06 in the presence of 100 mM sodium chloride. Thus, there was no consistent modulation by sodium of amiloride interaction with platelet α_2 receptors although an increased affinity for amiloride in the presence of sodium was consistently observed in studies with renal membranes.

Sodium did not modulate renal α_1-adrenergic receptor interactions with amiloride; receptor affinity for amiloride was not changed (Fig. 7) by the presence of sodium, although agonist affinity was slightly reduced as previously observed (8). Gpp(NH)p lowered receptor affinity for agonist while having no effect on amiloride binding to renal α_1- or to BC3H-1 β_2-adrenergic receptors.

Amiloride enhances radioligand dissociation at renal α_2-adrenergic receptors. To confirm that the effect of sodium on α_2 receptor affinity for amiloride was due to an allosteric effect, we determined the rate of dissociation of [^{3}H]rauwolscine from renal membranes in the presence and absence of amiloride. As shown in Fig. 8, the $t_{1/2}$ for dissociation shortened from 10.7 ± 0.6 min in the control to 8.5 ± 0.5 min in the presence of 300 μM amiloride ($p < 0.001$, paired one-tailed *t* test, $N = 5$); the change in rate of dissociation was apparent at the earliest time measured.

Discussion

Amiloride, a competitive inhibitor of membrane-associated sodium-binding sites (5), also binds to adrenergic receptors. As we show here, amiloride binds to adrenergic receptors in a variety of cells and tissues including renal cortical membranes, BC3H-1 cell membranes, and platelets. Amiloride binds to a single class of sites at α_1- and β-adrenergic receptors as indicated by pseudo-Hill slopes of approximately 1 (Figs. 3 and 7). This binding is competitive, as indicated by an increase in the apparent radioligand K_d, without an effect on the maximal number of binding sites. These results were obtained in both renal and BC3H-1 cell membranes. Binding to a single class of sites is characteristic of antagonist binding at adrenergic receptors unless subtype-selective agents are used to compete with nonselective radioligands. The results for α_1- and β-adrenergic receptors suggest that amiloride binds in a competitive, mutually exclusive manner with radioligand at the receptor-binding site.

The interaction of radioligand and amiloride with α_2-adrenergic receptors is complex. Amiloride appears to bind to more than one site on these receptors. This conclusion is supported by three pieces of evidence: 1) binding isotherms, 2) competition binding studies, and 3) kinetic analysis of ligand dissociation rate. Each of these will be discussed below.

In the presence of increasing concentrations of amiloride there is a dose-related increase in K_d and a reduction in the apparent number of binding sites for α_2-adrenergic receptors (Figs. 4 and 5); this is not compatible with a simple competitive interaction. The decrease in B_{max} together with an increase in K_d suggests that amiloride binds at two sites on the receptor; one site is likely to be the radioligand-binding site and the second site is presumably an allosteric site. Amiloride was therefore useful in revealing an additional site on α_2 receptors that was not apparent on either α_1- or β-adrenergic receptors.

Measurement of the pseudo-Hill slope in competition binding studies is commonly used to assess whether a ligand is interacting with a homogeneous class of sites (16). In contrast to α_1- and β-adrenergic receptors, where amiloride competed with a single class of sites (pseudo-Hill slope = 1), competition binding studies with amiloride at α_2 receptors consistently yielded pseudo-Hill slopes from 0.6 to 0.9 in both platelets and renal cortical membranes. This finding also suggests that amiloride interacts with more than one site on α_2-adrenergic receptors.

If amiloride were acting at an allosteric site on α_2-adrenergic receptors, the rate of ligand dissociation ought to be increased in the presence of amiloride. Indeed, 300 μM amiloride increased the rate of dissociation of [^{3}H]rauwolscine from renal cortical membranes. Although it is not possible to estimate the fractional occupation of amiloride at each of the two sites, we propose that the affinity of the allosteric site for amiloride is probably lower than at the radioligand-binding site; this difference in affinity might account for our inability to detect a

substantial change in dissociation rate in the presence of 100 μM amiloride (data not shown).

We hypothesize that the allosteric site to which amiloride binds is a sodium-binding site because amiloride is known to bind to several types of sodium recognition sites (1–3, 5) and sodium is known to affect the affinity of α_2-adrenergic receptors (11, 15, 17–21). The ability of sodium to enhance the affinity of the radioligand-binding site for amiloride in renal cortical membranes (Fig. 6) indicates that sodium and amiloride may interact at a common allosteric site. The difficulty in consistently observing an effect of sodium on amiloride's interaction with platelet α_2-adrenergic receptors may represent differences in the two tissues. In particular, this discrepancy may indicate differences in affinity of the allosteric site for amiloride or sodium in renal cortical α_2- compared to platelet α_2-adrenergic receptors.

The data presented here indicate that α_2-adrenergic receptors possess a site for allosteric regulation by sodium ions. Occupation of the allosteric site by amiloride decreases affinity of the receptor-binding site for radioligand by enhancing the dissociation rate. Occupation of the allosteric site by sodium may increase the affinity of the receptor-binding site for amiloride. We postulate that these effects are due to a conformational change of the receptor induced by binding to the "sodium site" and that amiloride, by virtue of its specificity for sodium-binding sites can reveal the "sodium site" on α_2-adrenergic receptors. We believe this is an allosteric site on the α_2 receptor as opposed to a site on a separate membrane component. This conclusion is consistent with results showing that sodium reduces agonist affinity of solubilized platelet α_2-adrenergic receptors or partially purified receptors from porcine brain (20, 21). The ability of sodium to enhance receptor affinity for amiloride demonstrates that amiloride does not exert effects on the α_2 receptor exclusively by binding to the sodium site. Moreover, the independent actions of sodium and guanine nucleotides support the hypothesis that the effects of sodium and guanine nucleotides are mediated by different entities in the membrane (20). The lack of effects of guanine nucleotides on interactions of amiloride demonstrates that amiloride binds as an antagonist and not as an agonist.

The amiloride-promoted decrease in B_{max} at α_2-adrenergic receptors is difficult to explain. The complexity of the interactions that we hypothesize involves features that are difficult to examine experimentally. Thus, in binding to the two different sites that we propose, amiloride could allosterically increase its own binding to the ligand site and thereby may mask radioligand binding and decrease B_{max}.

Amiloride can be added to the increasing list of drugs, including verapamil and quinidine (22–26), that were initially characterized as binding to specific membrane-associated ion channels, yet have now been demonstrated to bind to multiple classes of adrenergic receptors. The overlapping specificities of these drugs for cation sites and adrenergic receptors perhaps point to some general structural features of these classes of target sites. Amiloride may prove useful in demonstrating additional properties of a cation-like binding site on α_2-adrenergic receptors. Moreover, this site may provide the basis for some form of modulation at this receptor and may distinguish it from other classes of adrenergic receptors.

Acknowledgments

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