appears more complicated than in the avian ciliary ganglion because of the presence of peripheral aminergic and "peptidergic" neurons (*18, 20*). The situation in Gasserian and spinal ganglia is still unclear (*15*).

A close relationship between adrenergic and cholinergic axons in the proximity of smooth muscles of several peripheral organs has been described (*21*), although the two types of axons, as in our case, do not form true synaptic junctions. There is circumstantial evidence for a peripheral mutual interaction between adrenergic and cholinergic terminals (*21*).

Obviously, pharmacological studies are necessary to establish the functional role of the adrenergic fibers in the ciliary ganglion as compared to the other tissues discussed here. Marwitt *et al.* (*2*) studied the effect of adrenergic blockers in the pigeon ciliary ganglion in vitro using extracellular recording. Only dibenzyline showed some effect in concentrations that did not impair nerve conduction. Knowledge gained by studies of our model might relate to situations in the central nervous system, for instance in the cerebellum (*22*).

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9. After treating the animals with nialamide and L-dopa, Ehinger (*5*) observed enhancement of the fluorescence of adrenergic baskets as well as the appearance of some fluorescent cells, whose pharmacohistochemical behavior is not typical for adrenergic neurons. During our extensive electron microscopic study, nerve cells with granulated vesicles comparable to the interneurons of sympathetic ganglia were never observed in the ciliary ganglion. We noticed, however, the presence of a considerable number of mast cells.
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## Cation Dependence of High-Affinity Angiotensin II Binding to Adrenal Cortex Receptors

Abstract. *The specific binding of monoiodinated angiotensin II by particulate receptors from the bovine adrenal cortex is enhanced by addition of sodium and potassium ions, but not other cations. In the presence of 140 millimolar sodium, increased uptake of angiotensin II by adrenal receptors is associated with the appearance of high-affinity binding sites with an association constant of* $2 \times 10^9$ *liters per mole.*

Specific receptors for angiotensin II have been described in particulate subcellular fractions from the bovine and rat adrenal cortex (*1, 2*). The majority of the angiotensin II receptor sites in bovine and rat adrenal cortex particles were found to be located in the plasma membrane fraction (*2*). Meyer *et al.* (*3*) came to a similar conclusion about the origin of the particulate angiotensin receptors derived from aortic smooth muscle cells. The octapeptide angiotensin II displays much greater avidity for adrenal cortex receptors than the decapeptide precursor, angiotensin I. Also, competitive binding studies performed in the presence of adrenal cortex particles and $^{125}$I-labeled monoiodoangiotensin II show a close correlation between adrenal binding-inhibition activity and biological potency in a variety of angiotensin fragments and analogs (*1, 2*).

During an investigation of angiotensin receptors in various target tissues of the hormone, we noted that specific binding of $^{125}$I-labeled angiotensin II to particulate fractions of bovine and rat adrenal cortex was influenced by the buffer composition, and especially by the cation content of the incubation medium. The experiments shown in Fig. 1 depict the basic finding, that sodium and potassium ions significantly increased the binding of angiotensin II to bovine adrenal cortex receptors, whereas the tris(hydroxymethyl)aminomethane ion showed no such effect. The increase in angiotensin II binding appeared to be saturable with respect to the cation concentration between 0 and 200 m*M*. At concentrations of sodium and potassium above 250 m*M*, the stimulation of binding gradually decreased and reached the original level, observed in the absence of both cations, at about 500 m*M*. The effects of sodium and potassium on angiotensin II binding were specific for these ions, and were not reproduced by rubidium, cesium, lithium, or magnesium; rather, some of these cations caused inhibition of angiotensin binding at high concentrations (Fig. 1B).

Particulate receptor preparations in-

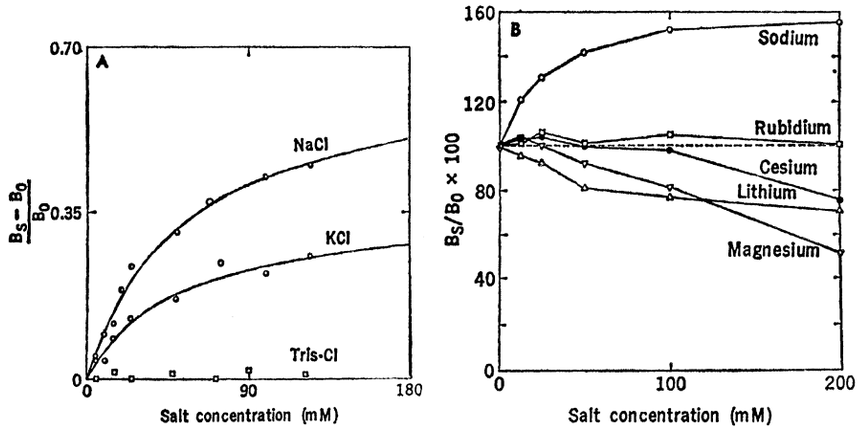


Fig. 1. (A) Influence of potassium, sodium, and tris-Cl on steady-state binding of angiotensin II by adrenal cortex receptors. The binding fraction was prepared from bovine adrenal cortex homogenates as previously described (*2*). Portions of the particulate receptor fraction (1.7 mg of protein per milliliter) were incubated with monoiodinated [$^{125}$I]angiotensin II (0.1 n*M*) in 50 m*M* tris-Cl buffer, *p*H 7.4, containing 0.3 mg of glucagon per milliliter, 5 m*M* dithiothreitol, and 0.1 percent heat-denatured bovine serum albumin, in a final volume of 250 μl. After 20 to 30 minutes, the incubated samples were diluted with 4 ml of ice-cold buffer and filtered through HAWP Millipore filters to separate "bound" and "free" angiotensin. $B_0$, bound angiotensin without salt, in the presence of tris buffer; $B_s$, bound angiotensin with cations present. Tris-Cl concentrations are expressed as excess over the 50 m*M* present in the basal incubation mixture. (Each point is a mean of triplicate determinations.) The specific activity of the [$^{125}$I]angiotensin II was determined by radioimmunoassay to be 700 to 1000 c/mmole. The adrenal binding fraction used here is enriched in adenylate cyclase (× 3), alkaline phosphatase (× 3), $Na^+$,$K^+$ adenosine triphosphatase (× 1.5), and angiotensin II receptor sites (× 4) compared to the original homogenate (*2*). (B) Effects of various cations (chloride form) on the steady-state binding of angiotensin II. Magnesium chloride at concentrations higher than 10 m*M* led to clumping of the particulate receptor preparation. Lithium chloride and cesium chloride (results not shown here) decreased the stimulation of angiotensin II binding by NaCl.

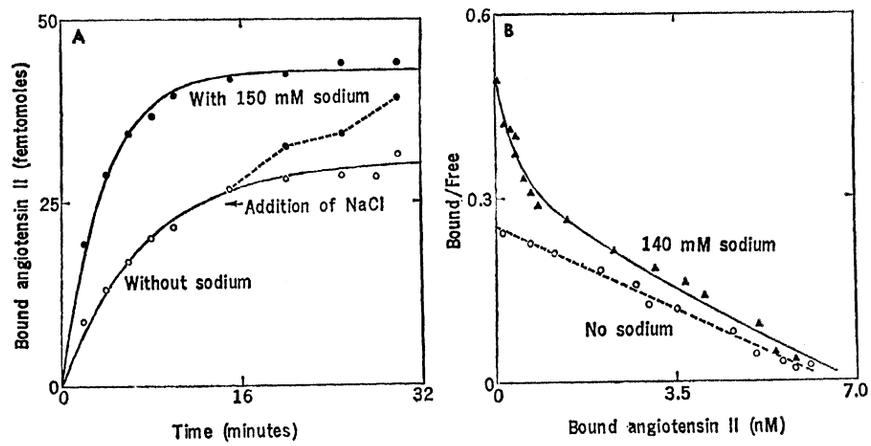


Fig. 2. (A) Time course of uptake of [$^{125}$I]angiotensin II (0.5 n*M*) by adrenal cortex particles (1.9 mg of protein per milliliter) incubated at 22°C in the presence and absence of 150 m*M* NaCl. The reaction was initiated by addition of ligand after equilibration of the samples for 3 minutes at 22°C. In a further series of samples, in which angiotensin II binding was initiated with NaCl absent, NaCl was added (10 μl to a final concentration of 150 m*M*) after 14 minutes (arrow), and the effect on subsequent binding was determined (dashed line). (B) Scatchard plot of steady-state binding data for angiotensin II incubated at 22°C for 30 minutes in the presence and absence of 140 m*M* NaCl. Incubation mixtures contained 0.2 percent heat-denatured bovine serum albumin, 0.3 mg of glucagon per milliliter, 5 m*M* dithiothreitol, 50 m*M* tris-Cl buffer (*p*H 7.4), and increasing concentrations of angiotensin II. Binding constants were calculated by computer analysis as previously described (*2*), and the data were plotted by a Calcomp plotter. A highly significant fit was obtained with two binding sites (of respective association constants $K_1$ and $K_2$ and concentrations $N_1$ and $N_2$) in the presence of NaCl. No high-affinity binding site was detected when experiments were performed in the absence of NaCl. Without NaCl, $K_1 = (3.9 \pm 0.1) \times 10^7\ M^{-1}$; $N_1 = 6.46 \pm 0.1$ n*M*. With 140 m*M* NaCl, $K_1 = (4.3 \pm 0.5) \times 10^7\ M^{-1}$; $N_1 = 6.6 \pm 0.3$ n*M*; $K_2 = 2.1 \pm 0.4 \times 10^9\ M^{-1}$; $N_2 = 0.2 \pm 0.01$ n*M*.

cubated in the presence of 150 m*M* NaCl showed an increased rate of uptake of angiotensin II throughout most of the time course compared to samples incubated without NaCl (Fig. 2A). When NaCl was added to the sodium-free samples, the rate of angiotensin binding increased and the quantity of bound peptide more rapidly approached the steady-state value achieved in samples incubated throughout with 150 m*M* NaCl. The presence of ouabain (3 m*M*), adenosine triphosphate (3 m*M*), and tyrosine (1 m*M*) did not influence the sodium-induced stimulation of angiotensin II binding.

Increased binding of the peptide hormone to its adrenal receptors could be due to an increase in the concentration or the affinity of binding sites, or alternatively to inhibition of peptide degradation. However, no significant effect of the presence of 150 m*M* NaCl on the degradation of free angiotensin II was demonstrable. Steady-state binding data showed, in the absence of sodium, only a single low-affinity binding site in adrenal cortex particles incubated with [$^{125}$I]angiotensin II at 22°C. With increasing concentrations of NaCl, high-affinity binding sites with an association constant $K_a$ of $2 \times 10^9\ M^{-1}$ appeared in the particulate receptor preparation, and were most clearly evident in the presence of 140 m*M* NaCl (Fig. 2B).

The dependence of high-affinity angiotensin II binding on sodium concentration suggests that an adjacent cation site may interact with the angiotensin II receptor to modify the uptake and actions of the peptide in the adrenal cortex. The effects of angiotensin II on papillary muscle (*4*) and smooth muscle in both guinea pig ileum (*5*) and blood vessels (*6*) are reportedly enhanced by increased sodium concentration, and it appears likely that sodium ions also influence angiotensin receptor affinity in these tissues. However, the reported effects of increased sodium levels on aldosterone secretion in response to angiotensin II have suggested that decreased sensitivity to angiotensin II occurs in the adrenal cortex. High serum sodium in the sheep (*7*) and a high sodium diet in the rat (*8*) have been shown to reduce the effect of angiotensin II on aldosterone secretion, in contrast to the enhancing effects of sodium on smooth muscle responses and binding affinity of adrenal receptor sites. Such an effect on aldosterone secretion by the adrenal glomerulosa cell must operate

on coupling or biosynthetic steps beyond the initial event of hormone-receptor interaction. Altered intracellular potassium concentrations during high sodium intake could also be an important factor in modifying the aldosterone secretory response of the adrenal cortex to angiotensin II.

In addition to the possible allosteric effect of a cation site on receptor affinity, other explanations for the action of sodium ions on angiotensin II binding in the particulate adrenal receptor preparation should be considered. Sodium ions may cause an alteration of the conformation of angiotensin II, or may stimulate an enzyme which catalyzes the breakdown of a compound inhibiting high-affinity angiotensin binding. In the experiments reported here, it was observed that the extent of the maximum stimulation of angiotensin II binding by sodium ions varied from as little as 10 percent to as much as 100 percent in different receptor preparations. Further investigation of this variability led to the demonstration that guanyl nucleotides also influence angiotensin binding, and are effective at nanomolar concentrations; these studies have been reported in detail elsewhere (*9*).

The dependence of angiotensin II binding to adrenal membranes on multiple factors, including sodium and potassium ions and guanyl nucleotides, indicates that modulation of the interaction between the peptide hormone and specific adrenal receptor sites is a complex process. It will be necessary to determine the extent to which these factors, which have been demonstrated to influence hormone uptake in vitro, are concerned in the regulation of angiotensin II binding and target cell activation in vivo.

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## Ethnic Classification of Mexican-Americans

In epidemiological studies as well as in other demographic or population studies, it is useful, and often essential, to make analyses within racial groups. The classification of racial hybrids, such as American Blacks and Mexican-Americans, however, may pose problems here. Thus Menck *et al.* (*1*), in their study of possible effects of air pollution on risk of lung cancer in Los Angeles County, claim to restrict their analysis to Caucasians but they include Mexican-Americans in this group. The problem here is that about one-third of the ancestry of Mexican-Americans in California is Mexican Indian, a fact which may be relevant to this and other epidemiological studies.

A good estimate of the amount of Caucasian ancestry in young Mexican-American adults residing in the Oakland, California, area in the 1960 to 1965 period can be made from published data (*2*). This estimate should be appropriate for Mexican-Americans of other urban areas of the state. The *r (cde)* gene of the Rh blood group system is especially useful here since it is essentially absent in "pure" Mexican Indians (*3*), but is common in Caucasians so that its frequency in the hybrid Mexican-Americans will reflect the amount of Caucasian ancestry (*M*, proportion of genes from Caucasian ancestors).

A maximum estimate of $M$ is obtained by assuming that the frequency of $r$ in the ancestral Mexican Indians was in fact zero, and that no other populations contributed genes (*4*). Then, with the use of the Oakland frequencies for $r$ of $0.3849 \pm 0.0039$ for persons of Caucasian ancestry (*5*) and $0.2615 \pm 0.0213$ for Mexican-Americans, and making the usual assumption (*6*) that population mixture was the only process determining the frequency of $r$ in modern Mexican-Americans, the calculated value of $M$ is $0.2615/0.3849 = 0.679 \pm 0.056$ (*7*). The minimum amount of Indian ancestry is therefore estimated to be $1 - M = 0.321 \pm 0.056$.

I have no information concerning the importance of this amount of Indian ancestry to the study of Menck *et al.*; it may well be negligible. My point is that the actual genetic composition of hybrid populations should be recognized and considered in analysis. Important ethnic differences in disease susceptibility exist, as Menck *et al.* recognize. It seems prudent to consider the possibility that biracial hybrids may also have distinctive disease susceptibilities.

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5. The frequency of $r$ in modern Spaniards, whose ancestors were presumably the major contributors of Caucasian genes, is within 1 percent of that of the Oakland Caucasians [A. E. Mourant, *The Distribution of Human Blood Groups* (Blackwell, Oxford, 1954), p. 384].
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7. $M \pm$ standard error of $M$. Standard error calculated by formula in (*6*).

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