Those observations are different from our results for which we used FcR-bearing monocytic cells and heat-stable IgG antibodies to HIV-1.

The presence of enhancing antibodies in the sera of individuals infected with HIV-1 indicates an immunological respone to HIV that may facilitate HIV-1 infection, instead of protecting the host. Conceptually such infection-enhancing antibodies may be more effective when neutralizing antibodies are absent or consumed by virus excess. Epidemiological investigations of the relation between enhancing and neutralizing antibodies and the clinical outcome of HIV-1 infection may determine the role of these antibodies in the progression of the infection. The presence of enhancing antibodies in HIV-1 infections will require careful consideration concerning the development of appropriate HIV vaccines. Further studies will be necessary to characterize the enhancing antibodies, including analysis of the isotypic distribution, the relevant class of FcR, and their relation to neutralizing antibodies and to the antibodies involved in lysis of virus-infected cells. It is particularly important to identify the immunogenic epitopes that react with the enhancing antibodies.

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Mineralocorticoid Action: Target Tissue Specificity Is Enzyme, Not Receptor, Mediated

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Mineralocorticoid receptors, both when in tissue extracts and when recombinant-derived, have equal affinity for the physiological mineralocorticoid aldosterone and for the glucocorticoids cortisol and corticosterone, which circulate at much higher concentrations than aldosterone. Such receptors are found in physiological mineralocorticoid target tissues (kidney, parotid, and colon) and in nontarget tissues such as hippocampus and heart. In mineralocorticoid target tissues the receptors are selective for aldosterone in vivo because of the presence of the enzyme 11β-hydroxysteroid dehydrogenase, which converts cortisol and corticosterone, but not aldosterone, to their 11-keto analogs. These analogs cannot bind to mineralcorticoid receptors.

THE ADRENAL CORTEX IS THE ONLY source of physiological glucocorticoids (cortisol and corticosterone) and mineralocorticoids (aldosterone). Classically, glucocorticoid receptors have been studied by determining the binding of the synthetic radiolabeled ligands, [^{3}H]dexamethasone and [^{3}H]triamcinolone acetonide, because these ligands have both high affinity for receptors and low affinity for transcortin, the plasma corticosteroid binding globulin. High-affinity sites that bind [^{3}H]aldosterone, the physiological mineralocorticoid, occur in a wide variety of tissues—both classical mineralocorticoid target tissues (for example, kidney, parotid, and colon) and others (for example, hippocampus and heart) (*1*). These sites, in the absence of transcortin, show equal affinity in cytosol preparations for aldosterone, cortisol, and corticosterone (the physiological glucocorticoid in mice and rats) and have been termed type I receptors, to distinguish them from type II classical dexamethasone-binding glucocorticoid receptors. In vivo, however, whereas injected [^{3}H]corticosterone and [^{3}H]aldosterone are similarly taken up and retained in the hippocampus and heart, the classical mineralocorticoid target tissues are selective for aldosterone and show little [^{3}H]corticosterone binding (*2*). The recombinant human renal mineralocorticoid receptor has been expressed and its binding specificity determined; just as for cytosol receptors, the human mineralocorticoid receptor in vitro has equal affinity for aldosterone, corticosterone, and cortisol (*3*). Given this equal receptor affinity and the much higher circulating levels of cortisol or corticosterone than aldosterone, additional mechanisms must operate to allow selective mineralocorticoid action in mineralocorticoid target tissues. From our studies (*2*) comparing in vivo with in vitro binding of [^{3}H]aldosterone and [^{3}H]corticosterone, it appears that this selectivity is the result of the exclusion of injected [^{3}H]corticosterone from receptors in mineralocorticoid target tissues.

Stewart *et al.* have proposed (*4*) a mechanism to account for the hypertension and Na^+ retention in patients with the syndrome of apparent mineralocorticoid excess, a syndrome associated with the congenital absence of the enzyme 11β-hydroxysteroid dehydrogenase (*5*). These patients are unable to convert cortisol to cortisone in their kidneys; therefore they have much higher than normal ratios of urinary cortisol to

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cortisone metabolites, and presumably much higher than normal renal cortisol levels. Stewart *et al.* proposed (*4, 6*) that these high intrarenal cortisol levels may overcome the normal specificity-conferring mechanisms that allow selective aldosterone binding in the kidney. Our studies suggest that the action of 11β-hydroxysteroid dehydrogenase in mineralocorticoid target tissues is the physiological mechanism that excludes cortisol and corticosterone from the nonselective receptors, thus allowing aldosterone to act as a specific mineralocorticoid.

If 11β-hydroxysteroid dehydrogenase confers aldosterone specificity in mineralocorticoid target tissues, then the enzyme must be selectively expressed in those target tissues but not in other organs containing type I receptors. To test this proposition, 1-day-adrenalectomized, 9-day-old rats were killed, and their hippocampus, kidney, parotid, colon, and heart were assayed for 11β-hydroxysteroid dehydrogenase activity by monitoring the conversion of [^{3}H]cortisol to [^{3}H]cortisone in vitro. There was no discernible activity in hippocampus and heart, as indicated by a single peak of [^{3}H]cortisol (F) at both zero time and after 2 hours of incubation (Fig. 1A). In contrast, the classic mineralocorticoid target tissues consistently showed conversion of [^{3}H]cortisol to [^{3}H]cortisone (E) after 2 hours. Under the conditions of study, the activity appears highest in the kidney, next highest in the parotid, and lowest in the colon.

In the next experiments, animals were given carbenoxolone 33 and 3 min before being killed (Fig. 1B); the tissues were then minced and incubated with [^{3}H]cortisol for 2 hours at 37°C. We chose the antiulcer drug carbenoxolone as the 11β-hydroxysteroid blocker because its parent compound glycyrrhetinic acid alters urinary cortisol to cortisone ratios in man (*6*), because the mineralocorticoid side effects of carbenoxolone are more pronounced than would be expected from its relatively low affinity for mineralocorticoid receptors (*7*), and because in preliminary studies in vitro the compound was effective in blocking the conversion of cortisol to cortisone by kidney minces (*8*). As might be expected, after treatment with carbenoxolone there was still no activity seen in hippocampus and heart (Fig. 1B). In the other tissues, however, treatment with carbenoxolone was followed by a substantial (kidney), near complete (parotid), or complete (colon) reduction in conversion of [^{3}H]cortisol to [^{3}H]cortisone, compared with control animals (Fig. 1A).

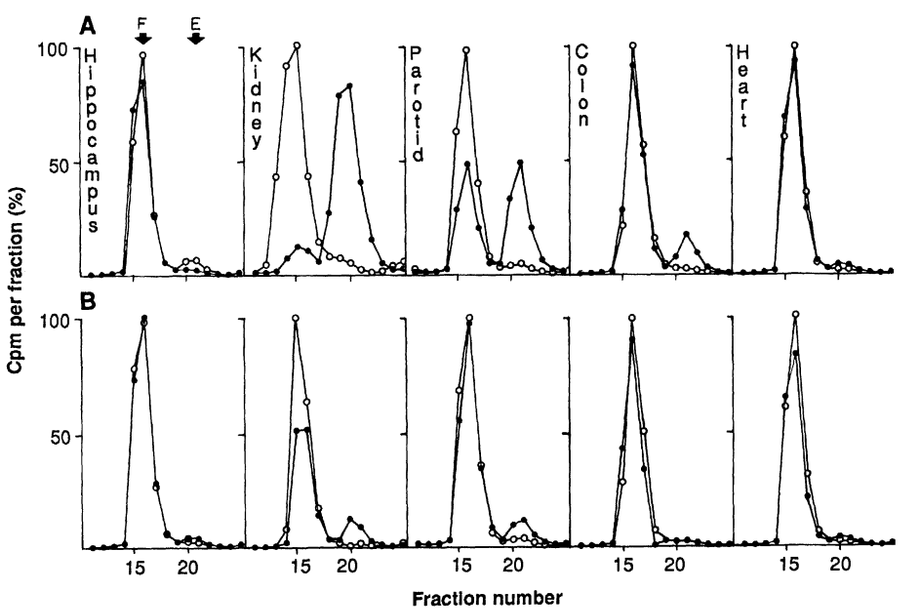

Fig. 1. (**A**) Analysis of in vitro conversion of [^{3}H]cortisol (F) to [^{3}H]cortisone (E) by tissue minces by high-performance liquid chromatography (HPLC). Tissues from 9-day-old, 1-day adrenalectomized rats were pooled from four animals, minced by hand with scalpel blades, and up to 0.5 g was incubated for 2 hours at 37°C in 2 ml of phosphate-buffered saline with ~200,000 cpm of [^{3}H]cortisol. At the end of the incubation, the incubates were shaken vigorously with 10 ml of ethyl acetate; the ethyl acetate was then removed by evaporation, and the [^{3}H]steroid taken up in ethanol and diluted with 0.1% trifluoroacetic acid (TFA) before HPLC analysis. Samples were injected onto a radially compressed CN reversed-phase column, and eluted isocratically with a solution of 16% acetonitrile and 0.08% TFA (1 ml/min), with the elution profile monitored at 214 nm. Fractions (0.5 ml) were collected for 20 min. Authentic cortisol eluted in fractions 15 to 17, and cortisone in 20 to 22. Shown are values for zero time incubation (open symbols) and a 2-hour incubation (solid symbols). Data shown are representative of three separate experiments on pooled tissues from 9- to 10-day-old adrenalectomized rats; similar profiles were also seen in tissues from individual mature rats. (**B**) Analysis of the effect of in vivo carbenoxolone-treatment on in vitro conversion of [^{3}H]cortisol to [^{3}H]cortisone by tissue minces by HPLC. Nine-day-old, 1-day adrenalectomized rats were injected subcutaneously with 10 mg of carbenoxolone 33 and 3 min before being killed by decapitation, and the tissues were removed for study. The procedures were as in (A). Data shown are from a single study done in parallel with the control study shown in (A) and are representative of two separate studies on carbenoxolone-treated 9- to 10-day-old rats.

In a parallel series of studies, rats (9 to 10 days old, 24 hours after adrenalectomy) were injected with [^{3}H]aldosterone or [^{3}H]corticosterone, either alone or with injection of carbenoxolone both 30 min before and concurrently with the tritiated tracer steroid; tissues were removed for analysis 15 min later. We chose 9- to 10-day-old rats, as in previous studies on in vivo binding, because they have little or no transcortin, thus making possible the use of [^{3}H]corticosterone as a ligand.

In hippocampus and heart (Fig. 2), there is essentially no difference between the binding of [^{3}H]aldosterone and [^{3}H]corticosterone in the presence or absence of carbenoxolone. In contrast, the mineralocorticoid target tissues show their typical selectivity for aldosterone in the absence of carbenoxolone (compare columns 1 and 4 in each set). In the presence of carbenoxolone, however, the level of [^{3}H]corticosterone binding markedly increases and approaches (colon) or equals (kidney and parotid) that of [^{3}H]aldosterone. In some tissues aldosterone binding appears slightly lowered by carbenoxolone, perhaps indicating some degree of direct competition for receptors at the concentrations used.

If corticosterone is excluded from mineralocorticoid receptors by the action of 11β-hydroxysteroid dehydrogenase, then the product of the reaction (11-dehydrocorticosterone) must have very low affinity for the aldosterone receptor. To test this proposition we used kidney cytosol from adrenalectomized rats as a source of mineralocorticoid (type I) receptors and thymic cytosol from the same rats as a source of classical glucocorticoid (type II) receptors, and we compared the ability of 11-dehydrocorticosterone and corticosterone to compete for receptor binding. Corticosterone ("B") and aldosterone (Aldo) competed equally for [^{3}H]aldosterone binding, as reported (*1*); 11-dehydrocorticosterone ("A"), in contrast, had only ~0.3% the affinity of its parent compound for type I receptors (Fig. 3A). A similar reduction in affinity for classical [^{3}H]dexamethasone (DM)-binding glucocorticoid receptors can be seen (Fig. 3B).

Our data suggest that 11β-hydroxysteroid dehydrogenase confers specificity in min-

eralocorticoid target tissues and allows aldosterone to occupy nonselective type I receptors in the presence of much higher circulating levels of glucocorticoids. In the physiologic glucocorticoids, the 11β-hydroxyl group is available for dehydrogenation, whereas the equivalent group of aldosterone is protected by the formation of 11,18-hemiketal or 11,18,20-hemiacetal bonds. Because 11β-hydroxysteroid dehydrogenase is a microsomal enzyme (*9*) it is not present in cytosol preparations. In such preparations the intrinsic nonselectivity of the type I receptors in mineralocorticoid target tissues becomes clear, a nonselectivity also shown in binding studies on the recombinant human mineralocorticoid receptor (*3*). In studies performed more than 15 years ago corticosterone was reported to have only 2 to 4% of the affinity of aldosterone for mineralocorticoid receptors in kidney slices (*10*).

Although our data indicate that the action of 11β-hydroxysteroid dehydrogenase is essential to explain the selectivity of mineralocorticoid action, they do not exclude roles for the enzyme in modulating steroid access to other receptors. Given the lower affinity of cortisone and dehydrocorticosterone for classical glucocorticoid receptors, cells in tissues with high levels of 11β-hydroxysteroid dehydrogenase activity may thus be relatively unresponsive to glucocorticoid. Whether this enzyme modulates the effective level of a glucocorticoid signal within and among tissues remains to be explored.

The recent demonstration that receptors for steroid hormones, thyroid hormones, and vitamin A derivatives are all members of a common supergene family attests to a remarkable degree of evolutionary versatility (*11*). Given this versatility, it is not clear why mineralocorticoid target tissue specificity is vested in an enzyme, rather than in the target tissue–specific expression of a mineralocorticoid receptor specific for aldosterone. One explanation may be that 11β-hydroxysteroid dehydrogenase activity is closely regulated in mineralocorticoid target tissues. If this is the case, then the extent to which type I receptors are occupied by 11β-hydroxysteroids is determined not only by their circulating free concentration, but also locally by the level of enzyme activity. Perhaps, then, the selection pressure for a nonspecific type I receptor reflects physiological circumstances—for example, during development or in response to stress—in which occupancy of type I receptors by glucocorticoids confers a survival advantage. There is ample evidence for changes in the activity of 11β-hydroxysteroid dehydrogenase in a variety of tissues over the course of development (*12*); it is as yet unknown whether there are also short-term changes in response to acute stimuli.

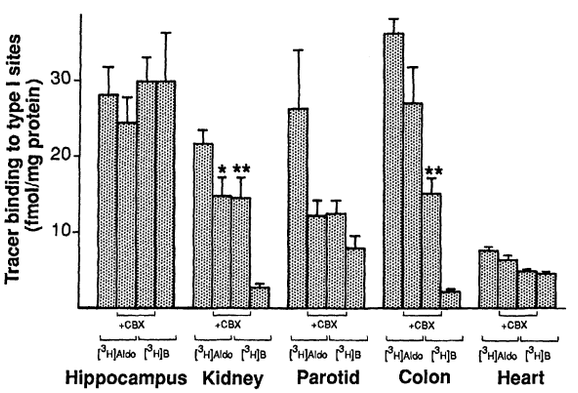

Fig. 2. Type I receptor binding of adrenal steroids in vivo; uptake and retention of [^{3}H]aldosterone ([^{3}H]Aldo) and [^{3}H]corticosterone ([^{3}H]B), and effect of treatment with carbenoxolone. Nine-day-old rats were adrenalectomized under ether anesthesia on the day before use and injected subcutaneously with 0.9 nmol of tritiated steroid per 100 g of body weight, plus a 500-fold excess of RU28362, with or without carbenoxolone treatment (10 mg subcutaneously 30 min before tracer injection, 1 mg with tracer). Excess of RU28362, a highly selective type II glucocorticoid receptor ligand, was used in all studies to exclude injected tracer from type II glucocorticoid receptors. In parallel studies, animals were injected with tracer, with and without carbenoxolone, and 100-fold excess of nonradioactive aldosterone or corticosterone to determine nonspecific binding, which was less than 0.7 fmol/mg of protein for either tracer in all tissues studied; all values shown represent specific (total minus nonspecific) binding. Animals were killed 15 min after tracer injection by decapitation, and blood was collected to monitor plasma levels of radioactive steroid. Tissues were dissected into ice-cold TM buffer (10 m*M* tris and 100 m*M* sodium molybdate), homogenized with a tissue homogenizer (Polytron P-10, Brinkmann), and the homogenates centrifuged for 1 hour at 105,000*g* in a refrigerated ultracentrifuge. Portions of the supernatant cytosols were shaken with an equal volume of hydroxylapatite slurry (0.5% w/v in phosphate buffer) to separate receptor-bound from other protein-bound or free steroid; the hydroxylapatite was washed twice, and the receptor-bound steroid was eluted into scintillation vials with ethanol. For each treatment for each tissue, $n = 6$. The values for hippocampus, kidney, parotid, and colon were obtained in one series of 48 rats (total and nonspecific binding), and those for heart were obtained, with kidney as a control (data not shown) in another series. Statistical comparisons were made by t test; $*P < 0.05$, $**P < 0.01$ compared with binding in the same tissue in absence of carbenoxolone.

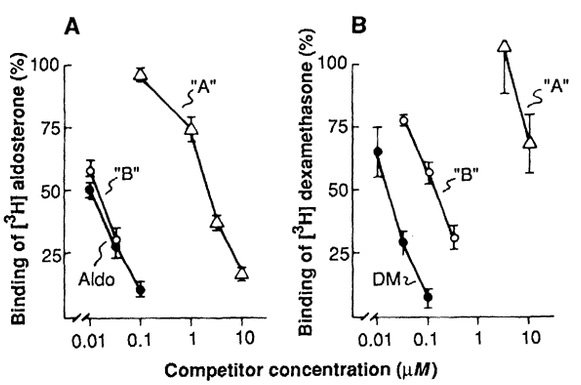

Fig. 3. In vitro binding of (**A**) [^{3}H]aldosterone in rat kidney cytosol and (**B**) [^{3}H]dexamethasone in thymus cytosol; competition by corticosterone and 11-dehydrocorticosterone. Mature (50-day-old) rats were adrenalectomized the day before use, tissues were homogenized in TM buffer, and cytosols were prepared as in the legend of Fig. 2. Portions of three separate cytosols were incubated overnight at 4°C with 10 n*M* tracer, and with increasing concentrations of nonradioactive aldosterone (Aldo), dexamethasone (DM), corticosterone ("B"), or 11-dehydrocorticosterone ("A"). Values shown are the means and SEM ($n = 3$) of duplicate determinations on three separate cytosols.

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