The Vasopressin-sensitive Adenylate Cyclase of the Rat Kidney

EFFECT OF ADRENALECTOMY AND CORTICOSTEROIDS ON HORMONAL RECEPTOR-ENZYME COUPLING*

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SUMMARY

A subcellular fraction prepared from rat kidney medulla contained vasopressin-sensitive adenylate cyclase and vasopressin binding sites. Vasopressin stimulation resulted in an increase in the maximal velocity of the reaction with no change in the apparent $K_m$ for ATP. A maximum activation ratio (5 to 10) was obtained at low Mg$^{2+}$ concentration (0.75 mM) and pH 7.4. The apparent $K_m$ for vasopressin was (1 to $7 \times 10^{-8}$ M). Vasopressin binding sites (0.3 pmole per mg of protein) can be identified with the hormonal receptors involved in adenylate cyclase activation on the basis of the following two criteria: (a) saturation of receptor sites and adenylate cyclase activation occurred in the same range of hormone concentrations and (b) a good correlation was observed between the relative potencies of unlabeled [8-arginine]vasopressin, [8-lysine]vasopressin, and oxytocin as inhibitors of [3H]vasopressin binding and as activators of the adenylate cyclase.

Adrenalectomy reduced the adenylate cyclase stimulation that was induced by vasopressin. It did not modify the basal enzyme activity or its activation by parathyroid hormone and sodium fluoride. The small reduction in vasopressin-binding capacity observed after adrenalectomy cannot account for the reduction in enzyme activation, an observation which suggests an impairment in the efficacy of receptor-enzyme coupling. Aldosterone treatment of adrenalectomized rats restored the hormone-binding capacity to control level but did not correct the receptor-enzyme coupling defect. Dexamethasone enhanced coupling efficiency in adrenalectomized rats and to a lesser extent in control animals. Dexamethasone was as active as aldosterone in restoring the hormone-binding capacity of adrenalectomized rats but had no effect in normal animals. Corticosterone was inactive under our experimental conditions. Neither adrenalectomy nor corticosteroid treatment modified the apparent $K_m$ of vasopressin for its receptor.

Aldosterone and dexamethasone were ineffective when added in vitro to the membrane preparation.

It is suggested that adrenal steroids exert a dual action on the vasopressin-sensitive adenylate cyclase of the kidney: (a) modulation of the number of receptor sites; this effect can be elicited by mineralo and glucocorticoids (aldosterone and dexamethasone) and (b) control of synthesis of a component enhancing the receptor-enzyme coupling efficiency.

In normal animals the receptor-enzyme coupling did not appear to be maximally stimulated.

It is now well established that neurohypophysial peptides exert their antidiuretic effect on the mammalian kidney through an increase in cyclic adenosine 3'5'-monophosphate production by target cells from the distal and collecting tubules (1-4). Stimulation of the membrane-bound adenylate cyclase of the renal medulla by antidiuretic hormone has been demonstrated in several species (4-17). More recently, it has been possible, with highly labeled [8-lysine]vasopressin, to characterize the hormonal receptors involved in the activation of the pig kidney adenylate cyclase (6, 7) and to define the relation between receptor occupancy and enzyme stimulation.

In this system, as in many others, very little is known about mechanisms of adenylate cyclase activation. Studies of ontogenetic development or possible physiological regulation of the receptor-adenylate cyclase system, or both, could help in elucidating some of the activation mechanisms. This would require the measurement of both binding of the hormone to its receptor sites and activation of the connected adenylate cyclase on the same preparation and under identical experimental conditions.

In rat adipose cells, Braun and Hechter (18) were able to demonstrate that adrenalectomy or hypophysectomy reduced, and that glucocorticoids restored, the responsiveness of the corticotropin-sensitive adenylate cyclase, whereas the effects of glucagon, epinephrine, and sodium fluoride were not modified. It was suggested that glucocorticoids induced the biosynthesis of a component required for corticotropin stimulation. It was not possible to decide if this component exerted its effect on the hormone-receptor interaction or on the coupling between receptor and adenylate cyclase. In this paper we show that corticosteroids are also necessary for maximal activation of adenylate cyclase from the renal medulla of the rat by antidiuretic hormone and that the major effect of corticosteroids is located at the receptor-cyclase coupling step.
EXPERIMENTAL PROCEDURE

Materials  Mice: Wistar rats (170 to 220 g body weight) were obtained from the Centre d'Embryologie, Centre National de la Recherche Scientifique, Orleans. Surgical adrenalectomy was performed under pentobarbital anesthesia. Control and adrenalectomized animals received a normal diet; adrenalectomized rats were given a 0.9% NaCl solution as a water source. Most of the experiments were performed 8 days after adrenalectomy. Corticosteroid treatments (aldosterone, dexamethasone, or corticosterone) started 5 days after adrenalectomy, each rat receiving 7 intramuscular injections at regular periods. The last injection was performed 2 hours before the animals were killed by decapitation. Blood was collected and the kidneys were rapidly excised and cooled in isotonic buffered sucrose solution at 0°C.

The medullary regions were separated from the cortical regions. Cortical or medullary tissues from the kidneys of two animals of each experimental group were pooled. The tissues were gently homogenized in 70 ml of a solution containing 5 mm Tris-HCl, pH 7.4, 3 mm MgCl2, 1 mm EDTA-Tris, pH 7.4, and 200 mm sucrose with an Elvehjem glass potter. A subcellular fraction was prepared as previously described (6, 7). The most important steps for the preservation of vasopressin stimulation of adenylate cyclase activity were an extensive washing of a low speed (600 X g) sediment of the homogenate in hypotonic medium (5 mm Tris-HCl, pH 7.4, 3 mm MgCl2, and 1 mm EDTA-Tris, pH 7.4). Except where otherwise specified, the experiments were performed immediately after membrane preparation.

The [3H]vasopressin (8-lysine-vasopressin) used (19) was labeled on the tyrosyl residue in position 2; its specific radioactivity was 12 Ci per mmole. It was radiochemically pure (19) and had retained 100% of the biological activity on rat vasopressor test (20) and adenylate cyclase activation of the pig kidney plasma membrane (6, 7).

Methods—Adenylate cyclase activity was measured by the conversion of [32P]ATP into labeled cyclic AMP. The incubation was 10 min at 37°C. In standard conditions, the incubation medium (total volume 100 μl) contained: 100 μM Tris-HCl, pH 7.4, 0.75 mm MgCl2, 0.25 mM ATP, 0.6 μCi of [32P]ATP, 1 mM cyclic AMP, 20 mM creatine-phosphate, 100 μg of creatine kinase, and 90 to 120 μg of membrane protein. The reaction was stopped by cooling and dilution of [32P]ATP with an excess of unlabeled perchloric acid. Cyclic AMP was separated by chromatography on dry aluminum oxide columns according to Ramachandran (21).

Details of the employed technique were previously described (22). Binding of [3H]vasopressin was measured after 15 min of incubation at 37°C in a medium identical with that used for the adenylate cyclase assay except for omission of the ATP regenerating system. Bound radioactivity was separated from the free hormone by filtration on Millipore filters (EAWP, 0.45 μm) as described for the pig kidney system (6, 7). The filters were washed three times with 10-ml aliquots of a solution kept at 0°C and containing 0.1% bovine serum albumin, 10 mm Tris-HCl, pH 7.4, and 1 mm MgCl2. Filters were dried and their radioactivity measured by liquid scintillation in 8 ml of the following medium: 50 mg of 1,4-bis[2-(4-methyl-5-phenoxazoyl)benzene and 4 g of 2,4-diphenyl ether in 1000 ml of toluene.

Properties of Adenylate Cyclase—Preparations from the medullary and cortical regions of the rat kidney contained an adenylate cyclase activity sensitive to vasopressin, PTH, and sodium fluoride (Table I). Basal and fluoride-stimulated activities are comparable in the cortex and the medulla. As first observed by Chase and Aurbach (9) the cortical enzyme is more sensitive to vasopressin than to PTH. The reverse effect is observed for the cortical enzyme. Maximal stimulations of the medullary activity by vasopressin and of the cortical activity by PTH (about 5-fold increase) approach that induced by sodium fluoride (10-3 M).

Measurements of basal and vasopressin (10-8 M)-sensitive adenylate cyclase activities from the renal medulla as a function of ATP concentration in the medium indicated that the hormonal stimulation was due to an increase in the maximal velocity of the reaction, with no change in the apparent affinity of the enzyme for ATP (apparent Km, 10-4 M) (Fig. 1). A slight optimum in the activation ratio was obtained at 10-3 M ATP. Decreasing the pH of the incubation medium to 6.5 nearly completely suppressed basal and vasopressin-sensitive adenylate cyclase activities. Maximal activities were obtained at pH 8.0 to 8.5; an optimum activation ratio was reached at pH 7.4 and remained unchanged at higher values. At constant ATP concentration (2.5 X 10-4 M), increasing Mg2+ concentration from 0.75 to 10 mm resulted in a linear increase in both basal and vasopressin-sensitive activities (Fig. 2); higher concentrations were inhibitory. The activation ratio was maximum at Mg2+ concentrations lower than 1.25 mm and thereafter decreased with higher concentrations. All further experiments were performed at 0.25 mM ATP, pH 7.4, and 0.75 mM Mg2+.

Storage of the enzyme preparation in liquid nitrogen resulted in decrease of approximately 60% in basal and hormone-stimulated activities; in addition, the apparent Km for vasopressin was increased. The addition of 10% dimethylsulfoxide during storage at 4°C did not prevent inactivation of the enzyme. In all experiments described below, freshly prepared enzymes were used to measure both adenylate cyclase and binding activities.

A significant adenylate cyclase activation by vasopressin was obtained at 10-8 M; the activation was maximal at 5 X 10-7 to 10-6 M, and the apparent Km for vasopressin was about 10-8 M. Variations were observed with regard to basal activity and maximal activation by vasopressin, PTH, or NaF.

Table I

<table>
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<th>Fraction</th>
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<td>Medulla</td>
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<tr>
<td>Cortex</td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>190</td>
</tr>
<tr>
<td>Vasopressin (10-8 M)</td>
<td>189</td>
</tr>
<tr>
<td>Parathyroid hormone (50 units/ml)</td>
<td>542</td>
</tr>
<tr>
<td>Sodium fluoride (10-8 M)</td>
<td>1100</td>
</tr>
</tbody>
</table>

Values are the mean of two determinations.

1 The abbreviations used are: cyclic AMP, cyclic adenosine 3':5'-monophosphate; vasopressin, [8-lysine]vasopressin; PTH, synthetic bovine parathyroid hormone.
FIG. 1 (left). Effect of different ATP concentrations on vaso-
pressin-sensitive adenylate cyclase of rat kidney medulla. Cyclic AMP production was measured for 10 min at 37" in the presence
($\Delta$) or absence of $10^{-8} \text{M}$ vasopressin (○). The incubation me-
dium contained 10 $\mu$M MgCl$_2$ and 50 $\mu$g of enzyme preparation.

Other assay conditions were as indicated under "Methods." The solid and dashed vertical lines indicate the apparent $K_m$ values for ATP under basal ($K_m = 9 \times 10^{-9} \text{M}$) and vasopressin-
stimulated conditions ($K_m = 8.1 \times 10^{-9} \text{M}$), respectively. The vaso-
pressin-stimulated over basal adenylate cyclase activities ratio
is indicated by ($\Delta$). Values are means of two experimental
determinations.

from one experimental series to the other. However (see Fig. 8),
enzyme preparations obtained at the same time from individual
animals of the same group always exhibited very similar activi-
ties. The variation ranges observed for all experimental series
reported in this paper were 90 to 380 pmoles of cyclic AMP per
10 min per mg of protein for the basal adenylate cyclase activities,
5 to 10 for the maximal activation ratio by vasopressin, and 1 to
$7 \times 10^{-8} \text{M}$ for the apparent affinity of the hormone.

Binding of $[^{3}H]$ Vasopressin to Rat Kidney Medulla Membranes—Rat kidney medulla membranes were able to bind vasopressin.
For a given concentration of labeled hormone, the amount of
bound hormone increased with time after its addition to the incu-
bation medium and reached an equilibrium value within 15 to
30 min (Fig. 3). Addition of a large excess ($10^{-5} \text{M}$)
of unlabeled hormone sharply reduced but did not completely suppress the
radioactivity binding. Determination of the inhibition of $[^{3}H]$-
vasopressin ($2.7 \times 10^{-8} \text{M}$) binding at equilibrium by increasing
amounts of unlabeled vasopressin (Fig. 4) indicated that a 54% inhibition is obtained at $5 \times 10^{-7} \text{M}$ vasopressin. Further addi-
tion of unlabeled peptide (up to $10^{-5} \text{M}$) did not modify the
magnitude of this maximal inhibition. Binding of vasopressin
can thus be described as the sum of two components: one cor-
responding to a limited number of high affinity binding sites and
a nonsaturable component. The latter most probably represents
a nonspecific adsorption of labeled hormone by the membrane
preparation. In all the binding assays, the total amount of
bound radioactivity was corrected by subtracting from each ex-
perimental value the nonspecific component measured by residual
$[^{3}H]$vasopressin binding in the presence of $10^{-5} \text{M}$ unlabeled
vasopressin.

Fig. 5 illustrates the evolution of total and nonspecific binding
as a function of $[^{3}H]$vasopressin concentration. The nonspecific
binding was negligible for concentrations lower than $5 \times 10^{-8} \text{M}$
and then increased linearly. The binding corresponding to the
saturable component was deduced from the difference between the
total and the nonspecific binding. Significant binding was
observed at $10^{-7} \text{M}$, and almost complete saturation occurred at a
concentration of $5 \times 10^{-8} \text{M}$, the apparent dissociation constant
being about $8 \times 10^{-9} \text{M}$. Since the nonspecific component rep-
resented a large fraction of the total binding, it was difficult to
obtain a precise determination of the maximal binding capacity
at high $[^{3}H]$vasopressin concentrations.

When the dose-dependent binding and the dose-dependent
adenylate cyclase activity were measured on the same enzyme
preparation (Fig. 6), it was observed that the progressive satura-
tion of the hormonal receptor sites occurred in the same range of
concentrations as for dose-dependent adenylate cyclase activa-
tion. Moreover, unlabeled [8-arginine]vasopressin, [8-lysine]-
vasopressin, and oxytocin were able to inhibit $[^{3}H]$vasopressin
binding. It appears from Fig. 7 that [8-arginine]vasopressin (the
natural antidiuretic hormone in the rat) is more active than
[8-lysine]vasopressin and oxytocin. A good correlation was ob-
protein) were exposed to \[3H\]vasopressin (3 \times 10^{-9} \text{M}) in the presence or absence of a large excess of unlabeled vasopressin (10^{-9} \text{M}). The incubation was performed at 37°C. The reaction was stopped by adding to each tube 2 ml of a cold solution containing 100 mM Tris-HCl, pH 7.4, and 1 mM MgCl₂. The amount of bound radioactivity was measured as a function of time. All experimental values were corrected for a blank value obtained after filtration of a control sample without membranes. The figure gives the time course of [3H]vasopressin binding in the absence (△) or presence (○) of 10^{-9} \text{M} unlabeled peptide as well as the difference between these two curves (●). Values are means of two determinations.

observed between the relative potencies of those three peptides to interact with the membrane receptor sites on one hand, and to activate the adenylate cyclase on the other.

A maximum inhibition of [3H]vasopressin binding was obtained with peptide concentrations in the incubation medium which gave a maximal adenylate cyclase activation. Finally, storage in liquid nitrogen resulted in a 4 fold decrease in receptor site binding capacity, a decrease similar to that observed for the adenylate cyclase activation (see above). These observations strongly suggest that the detected vasopressin binding sites are the hormonal receptors involved in adenylate cyclase activation.

Effect of Adrenalectomy on Vasopressin-sensitive Adenylate Cyclase of Rat Kidney—Since the eventual effects of adrenalectomy could only be deduced from the comparison of preparations obtained from different animals, it was necessary to exclude the possibility that the differences observed between control and experimental groups are not linked to fluctuations of the adenylate cyclase sensitivity to vasopressin or secondary effects of adrenalectomy (such as a modification of the membrane protein content used as a reference for the calculation of adenylate cyclase specific activities). As indicated above and illustrated by Fig. 8, preparations obtained from animals of the same group and tested during the course of the same experiment gave similar results. In addition, not only the vasopressin sensitive adenylate cyclase activity but also the basal value and stimulation by sodium fluoride and PTH were compared in control and adrenalectomized rats.

Fig. 8 compares the dose response curves to vasopressin of the adenylate cyclases prepared from four groups of two control or two 8-day adrenalectomized rats. Adrenalectomy clearly reduced the maximal activation by vasopressin but did not modify the apparent affinity for the hormone, whereas basal activity or activations by either sodium fluoride and PTH were unaffected.

Similar conclusions can be drawn from Table II in which the results of six experiments of the same type are given. The mean dose response curves to vasopressin (Fig. 9) indicate that the reduction by adrenalectomy of the maximal stimulation is about 30%. The mean apparent \(K_m\) values are very similar for control (3.7 \times 10^{-9} \text{M}) and adrenalectomized (3.6 \times 10^{-9} \text{M}) animals.

The effects of adrenalectomy were apparent 34 hours after operation and were maximal 8 days after (Fig. 10). No modification of basal activity was observed.

Effect of Adrenalectomy on Vasopressin Binding—In order to compare the maximal vasopressin binding capacity of kidneys from different groups of animals it is necessary to eliminate the possibility that variable amounts of endogeneous antidiuretic hormone could remain bound to renal receptors during the course of membrane preparation and thus could affect the apparent binding capacity deduced from [3H]vasopressin binding. Rats were anesthetized and perfused with 0.25% sodium chloride for 1 hour. They received intravenous [3H]vasopressin perfusion (1.3 pmoles per min per 100 g during 20 min).

The rate of labeled hormone administration corresponding to 10 times the normal secretion rate of endogeneous antidiuretic hormone, it can be reasonably assumed that, at the end of the perfusion, renal receptors are saturated with the labeled hormone at a specific radioactivity near the radioactivity of the administered [3H]vasopressin. At the end of the experiment the kidneys were immediately removed; the 600 × g pellet from the medullary regions was prepared as indicated above and its radioactivity measured. The maximal amount of vasopressin bound as calculated from radioactivity measurements was 0.6 ± 1.3 \times 10^{-12} \text{M} per mg of protein, i.e. less than 0.1% of the maximal binding capacity measured on membrane fractions. Thus, it seems very likely that, during the course of membrane preparation, bound endogeneous antidiuretic hormone is released from receptors and eliminated by the successive washings.

When vasopressin binding and adenylate cyclase activation were measured for both control and adrenalectomized rats, as a function of vasopressin concentration in the medium, it appeared from most experiments (Fig. 11) that adrenalectomy did not strongly modify the maximal binding capacity and affinity for the hormone of the receptor sites, although a significant reduction in adenylate cyclase activation was observed. In some other experiments, a small reduction in binding capacity and apparent affinity for vasopressin was observed in adrenalectomized rats. However, in each case, reduction in adenylate cyclase activation was more pronounced than reduction in binding. The mean difference between the percentage of decrease in adenylate cyclase activation and the percentage of variation in vasopressin binding was 10 ± 5, \(p < 0.01\) (18 paired determinations). These results suggest that the main defect in adenylate cyclase activation of adrenalectomized rats kidney is located at the receptor occupancy-enzyme activation step rather than at the hormone-receptor interaction step.

Effects of Corticosteroids on Adenylate Cyclase Activation and Vasopressin Binding—Eight-day adrenalectomized rats were treated with aldosterone (total dose: 0.175 mg in seven injections as indicated under “Methods” and the legends to Figs. 12 and 13), dexamethasone (total dose: 1.4 mg), or a combination of the two (0.175 mg of aldosterone and 1.4 mg of dexamethasone). Adenylate cyclase activation by vasopressin and vasopressin binding were measured on the same preparation. Each experimental series included control nontreated adrenalectomized rats. Aldosterone enhanced the maximal activation of adenylate cyclase by vasopressin but did not modify the apparent affinity.
for the hormone (Fig. 12). It also slightly increased the vasopressin binding. Due to technical limitations (see above discussion), it was not possible to accurately assess the effect of aldosterone treatment on the maximal binding capacity. However, when the relative increase in adenylate cyclase activation was compared to the relative increase in binding for the vasopressin concentrations used from $10^{-9}$ to $5 \times 10^{-6}$ M (Table III), it was apparent that the aldosterone effect could be accounted for by an increase in receptor capacity and in vasopressin-sensitive adenylate cyclase activity with no apparent modification in the receptor-enzyme coupling process.

Dexamethasone treatment led to a more pronounced effect (100% increase) than aldosterone on adenylate cyclase activation by vasopressin (Fig. 13). The effects of aldosterone and dexamethasone on vasopressin binding capacity were similar. Comparison of the relative increases in binding and adenylate cyclase activation due to dexamethasone treatment (Table III) clearly indicated that this corticosteroid significantly enhanced the coupling efficiency. The effects of aldosterone and dexamethasone were not additive (Fig. 13 and Table III).

As shown by Fig. 14, dexamethasone treatment of control nonadrenalectomized rats slightly enhanced the coupling efficiency as compared to that observed in untreated control nonadrenalectomized rats. When adrenalectomized rats were killed 2 hours after a single 0.2-mg intramuscular dexamethasone injection, no modification in adenylate cyclase responsiveness to vasopressin was observed as compared to control adrenalectomized rats.

Corticosterone treatment of 8-day adrenalectomized rats, by twice daily 0.8-mg intramuscular injections, was ineffective in correcting the effects of adrenalectomy on both adenylate cyclase activation and vasopressin binding (Table IV). On the other hand, neither aldosterone (10$^{-7}$ to 10$^{-4}$ M) nor dexamethasone (10$^{-7}$ to 10$^{-4}$ M) modified the responsiveness to vasopressin of the adenylate cyclase when added in vitro during the course of final enzyme activity assay (Table V).

**DISCUSSION**

In this paper we confirm the presence in the rat kidney medulla of a vasopressin-sensitive adenylate cyclase activity (13, 26). The basal specific activity of the enzyme as well as the magnitude of the maximal stimulation by [8-lysine]vasopressin are very similar to those measured in slightly different experimental conditions by Chase and Aurbach (9) and Douša et al. (13). The values are higher than those obtained on crude rat medulla homogenates by
Specificity of adenylate cyclase activation and vasopressin binding on rat kidney membranes. Adenylate cyclase activities (left panel) were determined as indicated under "Methods" in the presence of increasing amounts of [S-arginine]vasopressin (Δ), [S-lysine]vasopressin (●), or oxytocin (○). The incubation medium contained 89 μg of protein. Incubation was performed for 10 min at 37°. For vasopressin binding determinations, 188 μg of protein were incubated for 15 min at 37° in the presence of [3H]vasopressin (2 × 10⁻⁶ M) and increasing amounts of unlabeled [S-arginine]vasopressin (Δ), [S-lysine]vasopressin (●), or oxytocin (○). Experimental values were not corrected for nonspecific binding. Values are means of two determinations.

Lang and Edelman (27). We also confirm the observation by Chase and Aurbach (9) that the medullary adenylate cyclase is more sensitive to vasopressin than to PTH, the reverse situation prevailing for the cortical enzyme. The vasopressin binding sites detected on the rat kidney medulla have several properties similar to those of receptors previously characterized in pig kidney medulla plasma membranes (6–8). The maximal binding capacities are very comparable (0.2 to 0.4 pmole per mg of protein). The apparent dissociation constants for hormone-receptor interaction fell in the same range (about 10⁻⁷ M). These receptors are able to discriminate between structurally related neurohypophysial peptides.

Several correlations are observed between vasopressin binding and adenylate cyclase activation: (a) the progressive saturation of the binding sites occurs in a range of hormone concentration identical with that of the dose-dependent adenylate cyclase activation. The apparent Kₐ values deduced either from the labeled hormone binding curves or from the dose-adenylate cyclase activation curves are closely comparable. However, binding and adenylate cyclase activation curves are not superimposable. This could indicate as described for the pig kidney system the existence of a nonlinear receptor occupancy-enzyme activation relationship (7).

(b) The relative potencies of unlabeled [S-arginine]vasopressin, [S-lysine]vasopressin, and oxytocin as inhibitors of the binding of [3H]vasopressin closely correspond to their relative potencies as activators of the adenylate cyclase. From these observations, it seems reasonable to conclude that the detected vasopressin binding sites are the hormonal receptors involved in adenylate cyclase activation. Thus, measurements on the same preparation of both hormonal binding and adenylate cyclase activation make it possible to localize the effects of adrenalectomy or corti-
TABLE II
Effects of adrenalectomy on rat kidney medulla adenylate cyclase

For each of the six experiments described, the activities of enzymes prepared from control and 8-day adrenalectomized rats were compared. Adenylate cyclase was assayed as indicated under "Methods" in basal conditions or in presence of vasopressin (10⁻⁶ M), PTH (30 units per ml), or sodium fluoride (10⁻⁴ M). Activities are expressed as pmoles of cyclic AMP per 10 min per mg of protein. Values are the mean of two determinations.

<table>
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<tr>
<th>Experiment No.</th>
<th>Basal activity</th>
<th>Vasopressin (10⁻⁶ M)</th>
<th>PTH (30 units/ml)</th>
<th>NaF (10⁻⁴ M)</th>
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<td>Control</td>
<td>Adx</td>
<td>Adx/Control</td>
<td>Control</td>
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<td>95</td>
<td>96</td>
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<td>547</td>
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<td>Mean ± S.D.</td>
<td>0.99 ± 0.10</td>
<td>0.70 ± 0.06</td>
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*a Adx, adrenalectomized.

Fig. 9. Dose-response curves for vasopressin-sensitive kidney medulla adenylate cyclases from normal and adrenalectomized rats. The figure summarizes the results of six experiments in which control rats and 8-day adrenalectomized rats receiving a 0.9% NaCl liquid diet were compared. For each experiment the enzyme preparations were obtained and the adenylate cyclase activities measured in the same experimental conditions (see "Methods"). For a given set of determinations, all activities were expressed as the percentage of that obtained for control preparation in the presence of 10⁻⁶ M vasopressin. Values are mean ± S.D. The vertical lines indicate the apparent K₅₀ values for control (○) and adrenalectomized (O) animals.

corticosteroid deficiency reduces in some experiments the maximal vasopressin receptor-binding capacity. In all experiments the reduction in adenylate cyclase activity was more pronounced than the reduction in the receptor-binding capacity. The overall effect can be accounted for by: (a) a reduction in the number of receptor sites (and possibly of the number of the related adenylate cyclase molecules) and (b) a reduction in the efficiency of receptor-enzyme coupling.

Fig. 10. Evolution as a function of time after adrenalectomy of basal and vasopressin-sensitive adenylate cyclase activities. Twenty animals from the same group were used. Ten were adrenalectomized and given a 0.9% NaCl liquid diet. Five groups of two animals were killed together with the corresponding control animals 34 hours and 5, 8, 13, and 19 days after adrenalectomy. Adenylate cyclase activities were measured as indicated under "Methods," both in the absence (left panel) and presence (right panel) of 10⁻⁶ M vasopressin. For each paired determination the activity obtained from the adrenalectomized animals was expressed as the percentage of the activity obtained from corresponding control. Values are means of two determinations.

Clearly, adrenalectomy reduces the maximal activation of the adenylate cyclase by vasopressin with no modification of the apparent affinity for the hormone. This effect is very likely specific for the vasopressin-sensitive system since adrenalectomy did not affect the enzyme basal activity or its activation by sodium fluoride and PTH. This observation constitutes an additional argument for the dissociation between vasopressin- and PTH-sensitive adenylate cyclase systems. Incidentally, it can be noted that adrenalectomy also reduces the vasopressin-sensitive activity present in the renal cortex. The effect of adrenalectomy appears progressively with time, the maximal inhibition is obtained 8 days after surgery.

Corticosteroid deficiency reduces in some experiments the maximal vasopressin binding capacity. In all experiments the reduction in adenylate cyclase activation was more pronounced than the reduction in the receptor binding capacity. The overall effect can be accounted for by: (a) a reduction in the number of receptor sites (and possibly of the number of the related adenylate cyclase molecules) and (b) a reduction in the efficiency of receptor-enzyme coupling.

Aldosterone slightly increased the number of receptor sites in adrenalectomized rats but did not correct the defect in the receptor-enzyme coupling. In any case, the aldosterone effect is discrete. Lang and Edelman (27) reported that aldosterone treatment of adrenalectomized rats did not modify the adenylate cyclase response to an inframaximal vasopressin stimulation. On the other hand, Handler et al. (28) and Stoff et al. (29) clearly
demonstrated that the large increase in vasopressin-induced hydroosmotic response in aldosterone-treated toad bladders occurred after the cyclic AMP production step.

Dexamethasone like aldosterone slightly increases vasopressin binding capacity; in addition, the efficiency of the receptor-enzyme coupling is enhanced. This latter effect is predominant and accounts for the larger part of the increase in the vasopressin response.

Neither aldosterone nor dexamethasone were able to interact with the adenylate cyclase system when added in vitro to membrane preparations. By analogy with what is known on mechanism of action of corticosteroids (especially the action of dexamethasone on the corticotrophin-sensitive adenylate cyclase from rat adipose cells (Braun and Hechter (18)), it can be assumed that aldosterone and dexamethasone act on the kidney adenylate cyclase through control of protein synthesis. However, it cannot be excluded that these effects are secondary to modifications of renal (or other physiological) function(s) following adrenalectomy.

![Graph](http://www.jbc.org/)

**Fig. 11.** Comparative effects of adrenalectomy on adenylate cyclase activation and vasopressin binding. Kidney medulla membrane preparations were obtained from two control (●, △) and two 8-day adrenalectomized rats (○, □). Adenylate cyclase activation and vasopressin binding were determined as a function of hormone concentration in the incubation medium (see "Methods"). Adenylate cyclase activation (increase above basal value) is expressed as a percentage of maximal activation measured in the control preparation in the presence of $10^{-5}$ M vasopressin. Basal and maximal activities in the control preparation were, respectively, 93 and 582 pmoles of cyclic AMP per 10 min per mg of protein. Vasopressin binding is expressed as percentage of binding measured in the control preparation in the presence of $5 \times 10^{-9}$ M vasopressin (0.17 pmoles per mg of protein). Values are means of two determinations.

![Graph](http://www.jbc.org/)

**Fig. 12.** Effects of aldosterone in adrenalectomized rats on adenylate cyclase activation and vasopressin binding. Two groups of 8-day adrenalectomized rats were used. Both were given 0.9% NaCl as a liquid diet. Animals from one group (Adx + Aldo, □) received seven intramuscular injections of aldosterone (0.025 mg), 74, 64, 50, 40, 20, 10, and 2 hours before being killed. Animals from the other group (Adx, ○) were kept as controls. Adenylate cyclase activity (left panel) and vasopressin binding (right panel) were measured as a function of hormone concentration in the incubation medium (see "Methods"). Values are means of two determinations.
FIG. 13. Effects of dexamethasone plus aldosterone. The experiment was conducted as that described in Fig. 12. Treated animals received seven intramuscular injections of dexamethasone (0.2 mg) or of dexamethasone (0.2 mg), plus aldosterone (0.025 mg), 74, 64, 50, 40, 26, 16, and 2 hours before being killed. O, adrenalectomized rats (Adx); ■, adrenalectomized rats receiving dexamethasone (Adx + Dexa); and Δ, adrenalectomized rats receiving dexamethasone plus aldosterone (Adx + Aldo + Dexa).

**TABLE III**

**Effects of aldosterone and dexamethasone on [3H]vasopressin binding and adenylate cyclase activation**

The table is constructed from the experimental curves given in Figs. 12 and 13. For each of the vasopressin concentrations, the percentage of increase in vasopressin binding and adenylate cyclase activation due to corticosteroid treatment of adrenalectomized rats were calculated.

Values in the bottom of the table are mean ± S.D. These values were compared to 0 (Student's t test).

<table>
<thead>
<tr>
<th>Vasopressin</th>
<th>Aldosterone</th>
<th>Dexamethasone</th>
<th>Aldosterone + dexamethasone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>[3H]Vasopressin binding</td>
<td>Adenylate cyclase activation</td>
<td>[3H]Vasopressin binding</td>
</tr>
<tr>
<td><strong>M</strong></td>
<td><em>A</em></td>
<td><em>B</em></td>
<td><em>A</em></td>
</tr>
<tr>
<td>1 x 10^-9</td>
<td>13</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>3 x 10^-9</td>
<td>20</td>
<td>48</td>
<td>14</td>
</tr>
<tr>
<td>5 x 10^-9</td>
<td>25</td>
<td>37</td>
<td>30</td>
</tr>
<tr>
<td>1 x 10^-8</td>
<td>22</td>
<td>5</td>
<td>23</td>
</tr>
<tr>
<td>2 x 10^-8</td>
<td>23</td>
<td>29</td>
<td>28</td>
</tr>
<tr>
<td>5 x 10^-8</td>
<td>18</td>
<td>35</td>
<td>4</td>
</tr>
</tbody>
</table>

Mean ± S.D.

Mean difference (B - A)

<table>
<thead>
<tr>
<th><em>A</em></th>
<th>20 ± 4*</th>
<th>27 ± 17*</th>
<th>20 ± 10*</th>
<th>93 ± 51*</th>
<th>23 ± 35 NS</th>
<th>70 ± 17*</th>
</tr>
</thead>
</table>

* Significant at 95% probability levels.

* Significant at 99% probability levels.

* Not significant.

and corrected by corticosteroid treatment. If the aldosterone and dexamethasone effects are primarily located on the vasopressin renal target cells, this would indicate a dual regulation by corticosteroids of the adensinergic hormone-sensitive adenylate cyclase system: (a) a regulation exerted through modulation of the number of hormone receptor sites connected to adenylate cyclase molecules. The absence of effect on basal and fluoride-sensitive activities could indicate that the number of adenylate cyclase molecules is unaffected by adrenalectomy or corticoid treatments if one assumes that fluoride is able to maximally activate the adenylate cyclase (vasopressin and NaF are equally potent in enhancing medullary adenylate cyclase activity). On
**TABLE IV**

**Ineffectiveness of corticosterone in restoring vasopressin binding and adenylate cyclase activation in adrenalectomized rats**

Eight-day adrenalectomized rats received 0.8 mg of corticosterone injections twice daily. Membranes were prepared as indicated under “Experimental Procedure” and compared to preparations obtained from control nonadrenalectomized animals. The adenylate cyclase activity and vasopressin binding were measured as previously described in the presence of increasing amounts of hormone. For each hormonal concentration, vasopressin binding and adenylate cyclase activation measured in the corticosterone-treated animals were expressed as the percentage of the corresponding control value.

The mean values at the bottom of the table were compared to 100 (A and B) or 0 (difference B - A).

<table>
<thead>
<tr>
<th>Vasopressin (M)</th>
<th>Adrenaline, corticosterone treated, per cent of control</th>
<th>[3H]-Vasopressin binding (A)</th>
<th>Adenylate cyclase activation (B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 x 10^{-4} I</td>
<td>73</td>
<td>63</td>
<td></td>
</tr>
<tr>
<td>5 x 10^{-4} I</td>
<td>87</td>
<td>65</td>
<td></td>
</tr>
<tr>
<td>1 x 10^{-4} I</td>
<td>95</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>2 x 10^{-4} I</td>
<td>94</td>
<td>67</td>
<td></td>
</tr>
</tbody>
</table>

Mean ± S.D. (B - A) = -16 ± 3a

* Significant at 90% probability levels.

**TABLE V**

**Effects of dexamethasone and aldosterone added in vitro on rat kidney adenylate cyclase activity**

A membrane fraction was prepared from the kidney medulla of 8-day adrenalectomized rats.

Basal and vasopressin-sensitive adenylate cyclase activities were measured, as indicated under “Methods,” in the presence of increasing amounts of dexamethasone and aldosterone.

<table>
<thead>
<tr>
<th>Adenylate cyclase activity</th>
<th>Dexamethasone (M)</th>
<th>Aldosterone (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10^{-4}</td>
<td>10^{-6}</td>
</tr>
<tr>
<td>Basal</td>
<td>92</td>
<td>92</td>
</tr>
<tr>
<td>Vasopressin (10^{-9} M)</td>
<td>487</td>
<td>482</td>
</tr>
<tr>
<td></td>
<td>92</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>487</td>
<td>482</td>
</tr>
<tr>
<td></td>
<td>487</td>
<td>482</td>
</tr>
</tbody>
</table>

* Significant at 99% probability levels.

* Significant at 95% probability levels.

On the other hand, it is also possible that only a small fraction of the enzyme present in the renal medulla is in fact sensitive to vasopressin, the hormone being a more efficient stimulating agent than fluoride in this particular activity. In such a situation, a change in vasopressin activity may well be undetectable under basal or fluoride conditions.

(b) A second regulation could be exerted through a modulation of the receptor-adenylate cyclase coupling efficiency. The
latter effect being under the control of glucocorticoids only since aldosterone does not modify the receptor-occupancy and enzyme activation relationship. Distinct cytosol receptors for aldosterone and dexamethasone have been identified in rat kidney (30, 31). The results reported in this paper raise the possibility that a protein component may be directly involved in the sequence of events leading from binding of the hormone to its receptor to activation of the adenylyl cyclase. In this context, the study of genetic mutants in which the binding capacity could be preserved but the adenylyl cyclase activation absent would be of primary interest.

The observation that adrenalectomy led to only partial inhibition of the adenylyl cyclase response to vasopressin suggests that synthesis of receptors or the protein component involved in coupling, or both, proceeds at a lower rate in the absence of corticosteroids. On the other hand, the observation that dexamethasone has a positive effect in the control nonadrenalectomized animal indicates that in normal conditions the synthesis of the protein component does not proceed at its maximal rate.

In our experimental conditions, corticosterone (a natural circu- lating glucocorticoid in the rat) was ineffective in promoting an increase in vasopressin binding capacity and in receptor-enzyme coupling efficiency. Similarly, Braun and Hechter (18) found that cortisol is far less active than dexamethasone in enhancing the corticotropin response of adipose cell adenylyl cyclase from adrenalectomized rats. At present, there is no clear explanation for the lack of a significant corticosterone effect. This absence of activity can hardly be accounted for by very rapid elimination or destruction, since 2 hours after intramuscular injection of 800 μg of corticosterone to adrenalectomized animals, the plasma level was still 0.1 μg per ml as compared to 0.2 μg per ml in the normal, nonadrenalectomized, noninjected rat and 0.04 μg per ml in the adrenalectomized, noninjected animal (see also Dallman et al. (32)). On the other hand, two kinds of glucocorticoid receptors were demonstrated in the kidney, one having a highest affinity for dexamethasone than for corticosterone (Feldman et al. (30)); it is thus conceivable that dexamethasone and corticoste- rone have different effects.

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