Effect of Adrenal Steroid Hormones on the Response of the Toad's Urinary Bladder to Vasopressin

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A B S T R A C T This study was designed to examine the effect of adrenal steroid hormones on the response of the toad bladder to vasopressin. Aldosterone enhanced the short-circuit current response, the osmotic water flow response, and the urea permeability response to vasopressin. Since aldosterone also enhanced the short-circuit current response and the osmotic water flow response to adenosine 3',5'-monophosphate, the steroid effect on the bladder's response to vasopressin appears to be at a step beyond the stimulation of adenyl cyclase.

Indirect evidence was obtained that the effect of adrenal steroid hormones on the osmotic water flow response to vasopressin is mediated by a different hormone-tissue interaction than that mediating the effect of adrenal steroid hormones on sodium transport. In experiments with three different pairs of mineralocorticoid and glucocorticoid analogues, the former had a greater effect on short-circuit current, the latter on the osmotic water flow response to vasopressin. In addition, the spirolactone SC-14266 markedly inhibited the short-circuit current effect of dexamethasone and had little or no inhibitory effect on the dexamethasone enhancement of the osmotic water flow response to vasopressin. Aldosterone and dexamethasone stimulate the oxidation by the bladder of glucose-6-^{14}C and depress the rate of oxidation of glucose-1-^{14}C compared with glucose-6-^{14}C. SC-14266 inhibited the effect of dexamethasone on the oxidation of glucose-6-^{14}C but did not alter the effect of the steroid on the rate of oxidation of glucose-1-^{14}C compared with glucose-6-^{14}C, suggesting that the latter is a glucocorticoid effect and the stimulation of glucose-6-^{14}C oxidation a mineralocorticoid effect. Under conditions in which aldosterone has produced a marked enhancement of short-circuit current and the permeability response to vasopressin, the steroid had no detectable effect on cell water content or on cell sodium, potassium, or chloride.

INTRODUCTION

The effect of adrenal steroid hormones on the toad's urinary bladder has been the subject of considerable study since Crabbé (1) first reported that aldosterone stimulated sodium transport by this tissue in vitro. Evidence regarding specific receptors for the hormone, the role of protein synthesis in the response to the hormone, and the metabolic energy requirements for the aldosterone-induced sodium transport, have been the subject of recent reviews (2, 3). In this report evidence is presented that adrenal steroid hormones enhance the permeability response of the toad bladder to vasopressin and to adenosine 3',5'-monophosphate. This effect of adrenal steroid hormones appears to involve a different hormone-tissue interaction than that mediating the stimulation of sodium transport.

METHODS

Experiments were begun in the afternoon and concluded the following day. The protocol usually consisted of an

initial period of observation of sodium transport or the permeability response to vasopressin within a few hours of removal of the bladders from pithed toads. This was followed by overnight (14–16 hr) incubation of the bladder in Ringer's solution containing abundant (20 mM) glucose, with or without the steroid hormone or other agent being tested. After the long incubation, the Ringer's solution, now containing 5 mM glucose and the appropriate hormone or agent, was changed twice over a 1 hr period. During the next 2 hr, measurements of sodium transport and the permeability response to vasopressin were repeated. Paired tissue from the same bladder was used in all experiments.

Short-circuit current (SCC) was used to measure the rate of sodium transport (6). These experiments were performed in plastic chambers designed to isolate two areas of each hemibladder, thus yielding as many as four preparations from the same animal. SCC was measured by briefly short-circuiting the bladders at 30-min intervals. At the beginning of an experiment, an initial stable (less than 10% change in 30 min) short-circuit current (SCC_0) was obtained and used as a base line for evaluating the effect of agents on the SCC. The hormone or other agent being tested (and appropriate diluent) was added to the Ringer's solution on the mucosal and serosal surfaces of the bladder. The next morning, after the second change of solutions, SCC was again measured every 30 min over a 2 hr period. The mean SCC during this period ($SCC_{18-20 hr}$) was used to evaluate the effect of the experimental manipulation. In some experiments, the SCC response to 25 mU/ml of vasopressin or to 4 mM adenosine 3',5'-monophosphate (cyclic AMP) was evaluated at the end of this 2 hr period by measuring the SCC briefly every 5 min for 30 min after adding the neurohypophyseal hormone or cyclic AMP to the seroseal solution.

The diffusional permeability of the bladder to water (P_{H_2O}) and to urea (P_{urea}) was measured simultaneously by use of isotopic tracers (THO and urea-^{14}C). The same chambers were used in these experiments as in the SCC studies. Initial control permeability and the permeability response to vasopressin were evaluated within a few hours after removal from the toad. When the SCC stabilized, THO and urea-^{14}C were added to the solution bathing one surface of the bladder. Samples were taken from the solutions on both sides of the bladder after a 20 min equilibration period and again after an additional 30 min for estimation of control permeabilities. Vasopressin (25 mU/ml) was added, and 10 and 40 min later solutions were sampled to determine permeabilities during the 10–40 min period after the addition of vasopressin. Isotope and hormone were removed by changing the Ringer's solution twice over a 30 min period. Overnight incubation in 20 mM glucose solution, the addition of the steroid hormone, and the changes of solution the next morning were the same as in the SCC experiments described above. Control permeability and the permeability response to vasopressin were measured as on the previous afternoon. Radioactive samples (25 μl) were added to 14 ml of scintillation fluid (prepared by mixing 9 liters of toluene, 2 liters of Triton-X-100 (Packard Instrument Co., Inc., Downers Grove, Ill.), 60 g of 2,5-diphenyloxazole (PPO), and 1.1 g of 1,4-bis[2-(4-methyl-5-phenyloxazolyl)]benzene (POPOP) and counted in a Packard Tri-Carb dual channel counter. Internal standardization was used to obtain counting efficiencies.

The osmotic water flow permeability (P_{osm}) of the bladder was determined gravimetrically using the sac preparation of Bentley (7). The rate of water flow across the bladder from a 1:5 dilution of Ringer's solution on the mucosal surface to full strength amphibian Ringer's solution on the serosal surface was determined during an initial 30 min control period and during a 25 min interval starting 5 min after the addition of 30 mU/ml of vasopressin to the serosal solution. After measuring the initial osmotic permeability response to vasopressin (P_{osm_0}) the vasopressin was removed and the overnight incubation carried out as in the experiments in which diffusional permeabilities were measured. P_{osm} and the P_{osm} response to vasopressin were determined again, approximately 18 hr after excision of the bladder ($P_{osm_{18}}$).

The rate of oxidation of glucose-1-^{14}C and glucose-6-^{14}C was measured under two different conditions by dividing each bladder into four portions. Each portion was incubated in 3 ml of Ringer's solution in a 25 ml Erlenmeyer flask in a metabolic shaker. The composition of the Ringer's solution and the timing in these experiments were similar to those described above. The tissue was transferred to flasks containing the labeled substrates for the final hour of incubation at a time that would correspond to the 2 hr period during which the SCC_{18}, $P_{THO_{18}}$, $P_{urea_{18}}$, and $P_{osm_{18}}$ effects of the overnight incubation were measured in other experiments. At the end of the final 1 hr incubation, labeled carbon dioxide was released from the Ringer's solution by injecting H_2SO_4 into the sealed flask and was collected in 1 ml of Hyamine (Rohm & Haas Co., Philadelphia, Pa.) which was injected into a cup in the sealed flask. Appropriate blank flasks without tissue were run in each experiment. The Hyamine containing $^{14}CO_2$ and two samples of Ringer's solution containing each isotope were counted after addition to the liquid scintillation mixture, and internal standards were added to correct for quenching.

Tissue was incubated in 3 ml of Ringer's solution in 25-ml Erlenmeyer flasks in experiments in which the water and electrolyte composition of the bladder were measured. At least 45 min were allowed for equilibration with inulin-^{14}C. The analysis of the water and electrolyte composition of the bladders was performed as described previously (8) except that the dried, powdered tissue was extracted into 3.0 ml of 0.75 N HNO_3.

The Ringer's solution used in all experiments was composed of 90 mM NaCl, 25 mM NaHCO$_3$, 3 mM KCl, 1mM CaCl$_2$, 0.5 mM MgSO$_4$, 0.5 mM KH$_2$PO$_4$, equilibrated with 97%O$_2$, 3%CO$_2$. The Ringer's solution also contained 0.1 mg/ml of penicillin-G and 0.1 mg/ml of streptomycin. This solution had a pH of 7.6 and contained 220 mOsm/kg H$_2$O. All experiments were performed at 23–25°C.

Student's t test was used for statistical analyses. When ratios were evaluated, statistical tests were performed using the logarithm of each ratio. All experiments were performed in the winter and spring except where noted.

The vasopressin used was Pitressin, lot EF-103, Parke, Davis, & Co., Detroit, Mich. Aldosterone, corticosterone, 11-deoxycorticosterone (DOC), and adenosine 3',5'-monophosphate (cyclic AMP) were purchased from Calbiochem, Los Angeles, Calif. All isotopically labeled compounds were purchased from the New England Nuclear Corp., Boston, Mass. Dexamethasone was the gift of Dr. W. B. Gall of Merck, Sharp & Dohme, Rahway, N. J. SC-14266, potassium 3-(3-oxo-17β-hydroxy-4, 6-androstadien-17α-yl) propanoate, was the gift of Dr. L. M. Hoffman, G. D. Searle & Co., Chicago, Ill.

RESULTS

The effects of overnight incubation without hormone ("steroid depletion") and of incubation with aldosterone

on SCC and the SCC response to vasopressin and to cyclic AMP are summarized in Figs. 1 and 2. The results of these experiments may be expressed as the ratio of the SCC after prolonged incubation ($SCC_{18\ hr}$) to the SCC the tissue manifested when it first stabilized after removal from the toad (SCC_0). The effect of different experimental conditions can then be evaluated by the ratio of the $SCC_{18\ hr}/SCC_0$ for tissue incubated under one condition to the $SCC_{18\ hr}/SCC_0$ for tissue from the same bladder incubated under other conditions. The data in Fig. 1 indicate that tissue incubated for 18 hr with $2 \times 10^{-7}M$ aldosterone manifests a SCC five times that of paired tissue incubated for the same period without steroid hormone. In addition, the mean increment in SCC in response to a high concentration of vasopressin (25 mU/ml) was also five times greater in tissue treated with aldosterone. The enhancement by aldosterone of the SCC response to vasopressin is similar to that reported by Fanestil, Porter, and Edelman (9). Since cyclic AMP, the intracellular mediator of the action of vasopressin, also stimulates SCC in the toad bladder (10), the effect of aldosterone on the SCC response to cyclic AMP was examined (Fig. 2). Dominican toads were not responsive to cyclic AMP at the time of these experiments (May 1968), so Columbian toads were used without 0.6% NaCl pretreatment. Although the effect of aldosterone on SCC was not as striking as in Fig. 1, the response to cyclic AMP was clearly enhanced in bladders incubated with aldosterone compared with the response in paired "steroid-depleted" bladders. Thus it would appear that aldosterone enhances the SCC response of the bladder to vasopressin
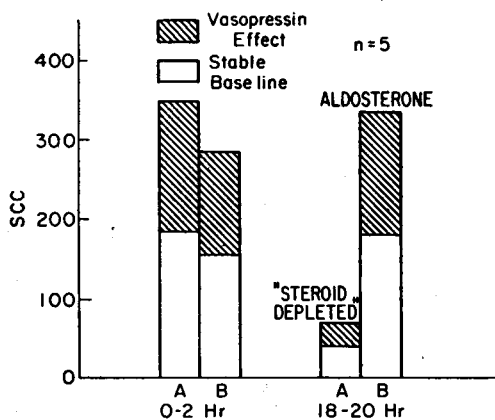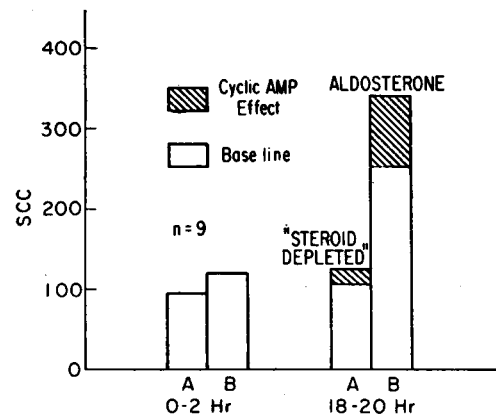

FIGURE 2   The effect of 2×10^{-7} M aldosterone on SCC and the response to 3',5'-adenosine monophosphate (cyclic AMP). In paired experiments the mean

$$\frac{\text{aldosterone } (18-20 \text{ hr})/(0-2 \text{ hr})}{\text{depleted } (18-20 \text{ hr})/(0-2 \text{ hr})}$$

was 1.89 ($P < 0.001$). The effect of cyclic AMP was tested only 18–20 hr. The mean effect corrected for base line SCC at 0–2 hr, $\dfrac{\text{aldosterone (cyclic AMP effect)}/SCC_0}{\text{depleted (cyclic AMP effect)}/SCC_0}$ was 4.39 ($P < 0.02$).

at a point beyond the adenyl cyclase catalyzed step of cyclic AMP production.

The effect of aldosterone on the osmotic water flow permeability response (P_{osm}) of the bladder to vasopressin is summarized in Fig. 3. Conditions were similar to those employed in the SCC experiments. Prolonged (18 hr) incubation in vitro resulted in a 40% reduction in the maximum P_{osm} response to vasopressin, whereas

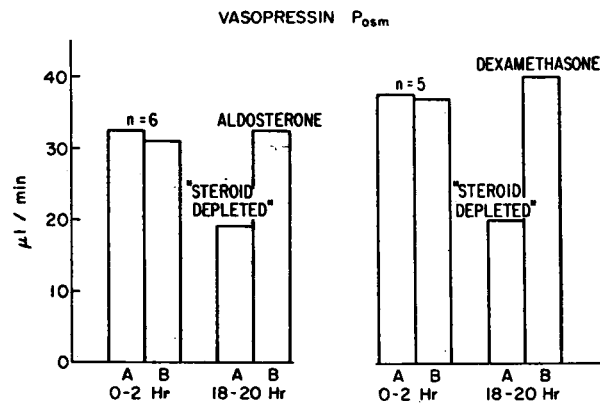FIGURE 1   The effect of 2×10^{-7} M aldosterone and "steroid depletion" on short-circuit current (SCC) and the SCC response to vasopressin. A and B are the two paired areas of tissue at 0–2 hr and at 18–20 hr. In paired experiments, the mean $\dfrac{\text{aldosterone } (18-20 \text{ hr})/(0-2 \text{ hr})}{\text{depleted } (18-20 \text{ hr})/(0-2 \text{ hr})}$ was 5.33 ($P < 0.001$) for the vasopressin effect. SCC is expressed as $\mu amp/3.2\ cm^2$.

FIGURE 3   The effect of 2×10^{-8} M aldosterone and 2×10^{-8} M dexamethasone on the osmotic water flow permeability (P_{osm}) response to vasopressin. In paired experiments, the mean P_{osm} response to vasopressin $\dfrac{\text{aldosterone } (18-20 \text{ hr})/(0-2 \text{ hr})}{\text{depleted } (18-20 \text{ hr})/(0-2 \text{ hr})}$ was 2.25 ($P < 0.02$), and $\dfrac{\text{dexamethasone } (18-20 \text{ hr})/(0-2 \text{ hr})}{\text{depleted } (18-20 \text{ hr})/(0-2 \text{ hr})}$ was 2.26 ($P < 0.01$).

bladders incubated with 2×10^{-8}M aldosterone or dexamethasone maintained the P_{osm} responsiveness they manifested shortly after removal from the toad. Aldosterone also enhanced the P_{osm} response of the bladder to 4 mM cyclic AMP compared with paired, "steroid-depleted" tissue (Fig. 4). The P_{osm} response of the bladder to cyclic AMP usually is less the second time it is tested with the nucleotide, as in the experiment on the left of Fig. 4, regardless of the interval between two test periods (11, 12). For this reason, in the experiment on the right of Fig. 4, the initial responsiveness of each sac was not tested. The effect of aldosterone was still evident and resembles the effect of the steroid on the SCC responsiveness to cyclic AMP (Fig. 2). In the entire study, there was no detectable effect of aldosterone or steroid depletion on P_{osm} in the absence of vasopressin or cyclic AMP.

Aldosterone also altered the permeability properties of the bladder measured by the rate of diffusion across the bladder of isotopically labeled urea (P_{urea}) or water (P_{H_2O}) without an osmotic gradient. In the experiments in Fig. 5, the bladders initially manifested a higher P_{urea} than reported elsewhere (13), although the increment in P_{urea} in response to vasopressin as well as P_{H_2O} were normal. The high P_{urea} values may be related to pretreatment of the toads in 0.6% NaCl. In any event, P_{urea} fell to very low values in steroid-depleted tissue and to a value in the generally reported range in tissue incubated with aldosterone. The change in P_{urea} in response to vasopressin was much lower in steroid-depleted
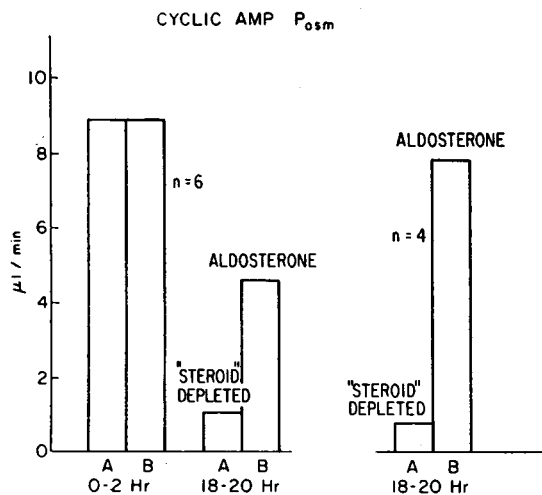

CYCLIC AMP P_{osm}

FIGURE 4 The effect of 2×10^{-8} M aldosterone on the P_{osm} response to cyclic AMP. In the experiment on the right, the response to cyclic AMP was not tested at 0–2 hr. In paired experiments, the mean P_{osm} response to cyclic AMP $\dfrac{\text{aldosterone } (18\text{–}20 \text{ hr})/(0\text{–}2 \text{ hr})}{\text{depleted } (18\text{–}20 \text{ hr})/(0\text{–}2 \text{ hr})} = 4.24$ ($P < 0.001$) and (aldosterone 18–20 hr)/(depleted 18–20 hr) = 9.5 ($P < 0.001$).
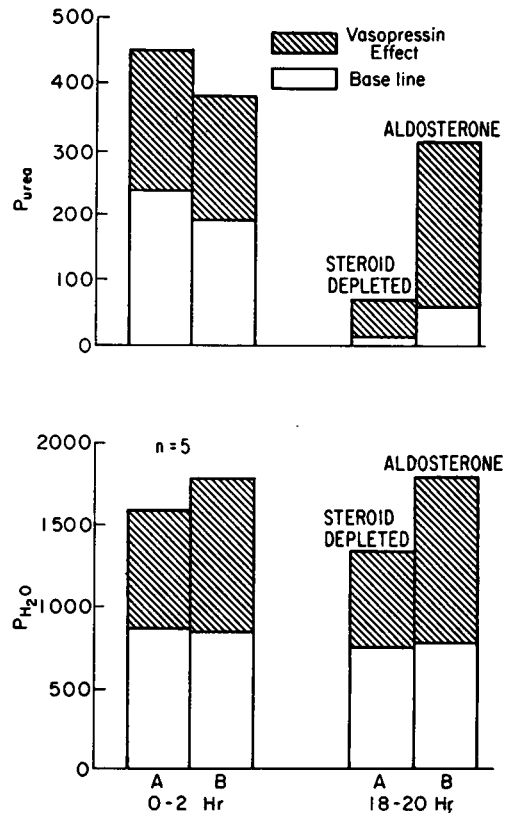
FIGURE 5 The effect of aldosterone on the permeability to urea (P_{urea}) (cm·sec^{-1}·10^{-7}) and water permeability (P_{H_2O}) (cm·sec^{-1}·10^{-7}) and the response to vasopressin. The mean ratio for the vasopressin effect $\dfrac{\text{aldosterone } (18\text{–}20 \text{ hr})/(0\text{–}2 \text{ hr})}{\text{depleted } (18\text{–}20 \text{ hr})/(0\text{–}2 \text{ hr})}$ for $P_{urea} = 7.80$ ($P < 0.005$) and for $P_{H_2O} = 1.23$.

tissue than in the same tissue initially or in paired aldosterone-treated tissue.[1] The slight increase in the P_{H_2O} respone to vasopressin in aldosterone-treated tissue was not statistically significant.

In the experiments in Figs. 1–5, the adrenal steroid was present throughout the prolonged incubation and prevented the loss of responsiveness to vasopressin. The ability of aldosterone to induce responsiveness in steroid-depleted tissue was tested by adding the hormone to tissue that had been incubated for 18 hr without

[1] In subsequent experiments with other groups of toads prepared in 0.6% NaCl or in tap water, in which the initial P_{urea} was low, a similar effect of aldosterone on the P_{urea} response to vasopressin was demonstrated. For example, in all experiments performed in July, with toads that were not pretreated with 0.6% NaCl, the mean base line P_{urea} values for control and experimental groups were 18 and 19 $\times$ 10^{-7} cm·sec^{-1}, and the mean ratio for the vasopressin effect,

$\dfrac{\text{aldosterone } (18\text{–}20 \text{ hr})/(0\text{–}2 \text{ hr})}{\text{depleted } (18\text{–}20 \text{ hr})/(0\text{–}2 \text{ hr})}$

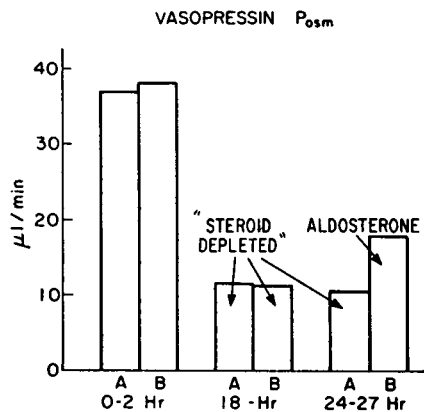for $P_{urea} = 3.47$ (n = 9, $P < 0.01$).

FIGURE 6 The effect of aldosterone on the P_{osm} response to vasopressin of steroid-depleted tissue. The mean ratio $\dfrac{\text{aldosterone } (24\text{--}27 \text{ hr})/(0\text{--}2 \text{ hr})}{\text{depleted } (24\text{--}27 \text{ hr})/(0\text{--}2 \text{ hr})} = 1.98$ (n = 13, $P < 0.02$).

hormone. In these experiments (Fig. 6), steroid-depleted tissue treated with 2×10^{-7}M aldosterone for 6–9 hr had a higher P_{osm} response to 25 mU/ml of vasopressin than paired depleted tissue, although the P_{osm} response to
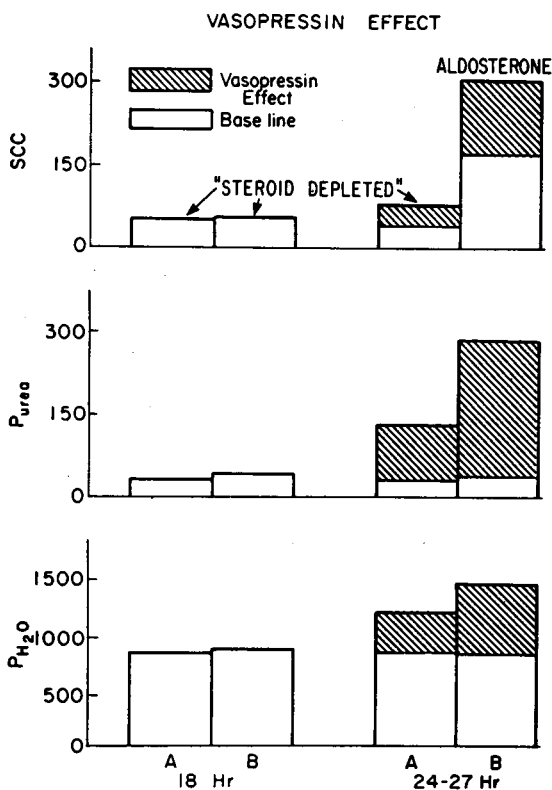

FIGURE 7 The effect of aldosterone on the SCC, P_{urea}, and P_{H_2O} and the response to vasopressin of steroid-depleted tissue. The mean ratios for the vasopressin effect aldosterone/depleted were SCC = 3.90 ($P < 0.001$), P_{urea} = 3.83 ($P < 0.005$), and P_{H_2O} = 2.61 ($P < 0.05$), n = 9.
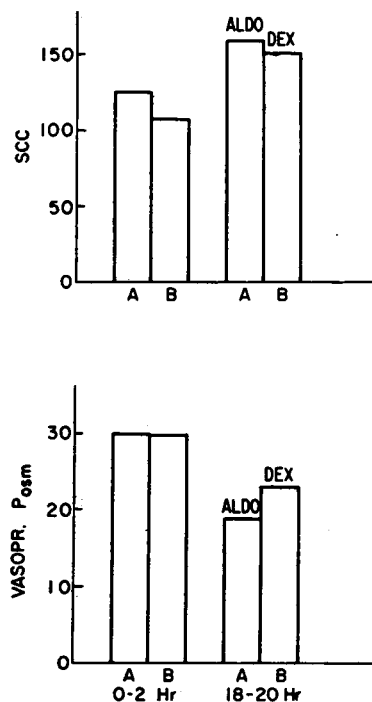

FIGURE 8 The effect of 2×10^{-9} M aldosterone and 2×10^{-9} M dexamethasone on the SCC of paired tissues and on the P_{osm} response to vasopressin of paired tissues. The mean ratio $\dfrac{\text{aldosterone } (18\text{--}20 \text{ hr})/(0\text{--}2 \text{ hr})}{\text{dexamethasone } (18\text{--}20 \text{ hr})/(0\text{--}2 \text{ hr})}$ for SCC = 1.06 (n = 6), and for the P_{osm} response to vasopressin = 0.81 (n = 7, $P < 0.005$).

vasopressin did not return to the high value observed initially at 0 hr. Aldosterone treatment of steroid-depleted tissue also resulted in a marked rise in SCC and the SCC response to vasopressin, as reported previously (9), and a rise in the P_{urea} and P_{H_2O} response to vasopressin (Fig. 7).

Since the P_{osm} response of the bladder to vasopressin is independent of sodium transport, the possibility was raised that the enhancement of the P_{osm} response to vasopressin and the stimulation of SCC by aldosterone might be the result of two separate effects of the steroid. In other words, stimulation of SCC might be a mineralocorticoid effect, and the enhancement of the P_{osm} response to vasopressin might be a nonmineralocorticoid or glucocorticoid type effect of aldosterone. This thesis was tested first by comparing the relative activity of three pairs of mineralo- and glucocorticoid hormones (aldosterone vs. dexamethasone, aldosterone vs. corticosterone, and DOC vs. corticosterone) on the P_{osm} response to vasopressin and on SCC. The hormones were employed in submaximal concentrations selected to yield equivalent effects either on the P_{osm} response to vasopressin or on SCC. In these experiments, aldosterone

and dexamethasone (Fig. 8) and DOC and corticosterone (Fig. 9) exerted the same stimulatory effect on SCC. However, in each study the glucocorticoid-type hormone (dexamethasone [Fig. 8] and corticosterone [Fig. 9]) had a greater effect than the paired mineralocorticoid on the P_{osm} response to vasopressin. In a complementary fashion, in studies in which aldosterone and corticosterone yielded equivalent effects on the P_{osm} response to vasopressin, aldosterone had a greater effect on SCC (Fig. 10).

Although the experiments in Figs. 8–10 are consistent with the idea that separate glucocorticoid and mineralocorticoid systems exist in the toad bladder, each system having a different affinity for each hormone analogue, the selectivity for each type of hormone is not great. Further support for the presence of two separate systems was obtained in experiments employing a spirolactone. These agents are thought to inhibit specifically the mineralocorticoid activity of adrenal steroid hormones by competing for receptor sites and have been shown to block the effect of aldosterone on the SCC of toad bladder (14). Were there separate glucocorticoid and mineralocorticoid systems, the spirolactone, by interacting with the mineralocorticoid receptor, would inhibit the SCC effect of adrenal steroid hormones and exert little or no effect on the enhancement of the P_{osm} response to vasopressin, a presumed glucocorticoid effect of the

FIGURE 10 The effect of 2×10^{-9} M aldosterone and 10^{-8} M corticosterone on the SCC of paired tissues and on the P_{osm} response to vasopressin of paired tissues. The mean ratio $\dfrac{\text{aldosterone } (18-20 \text{ hr})/(0-2 \text{ hr})}{\text{corticosterone } (18-20 \text{ hr})/(0-2 \text{ hr})}$ for SCC $= 1.69$ (n $= 8$, $P < 0.005$), and for the P_{osm} response to vasopressin $= 0.95$ (n $= 7$).

steroid hormone. Two series of experiments were performed, the first in the spring, and the second series in the fall. The effect of the spirolactone SC-14266 on the SCC response to dexamethasone in the first series is summarized in Fig. 11. In these experiments, in which four SCC areas were obtained from a single toad bladder, 5×10^{-6} M SC-14266 had no effect on SCC by itself but completely inhibited the SCC response to 3.5×10^{-9} M dexamethasone. In addition, the enhancement of the SCC response to vasopressin was also blocked by the spirolactone. In contrast to the marked effect of the spirolactone on the SCC effects of dexamethasone, in experiments performed at the same time the inhibitor was virtually without effect on the enhanced P_{osm} response to vasopressin induced by dexamethasone (Fig. 12). In P_{osm} experiments, each bladder yields only two areas, so separate paired experiments of control (steroid depletion) vs. SC-14266, control vs. $3.5 \times$
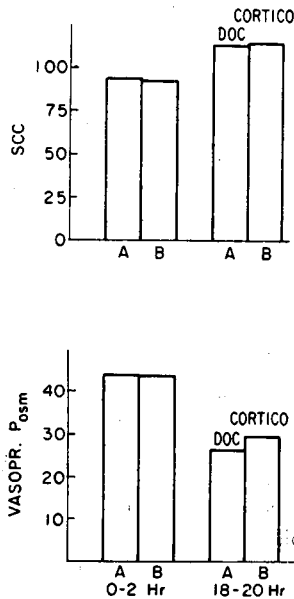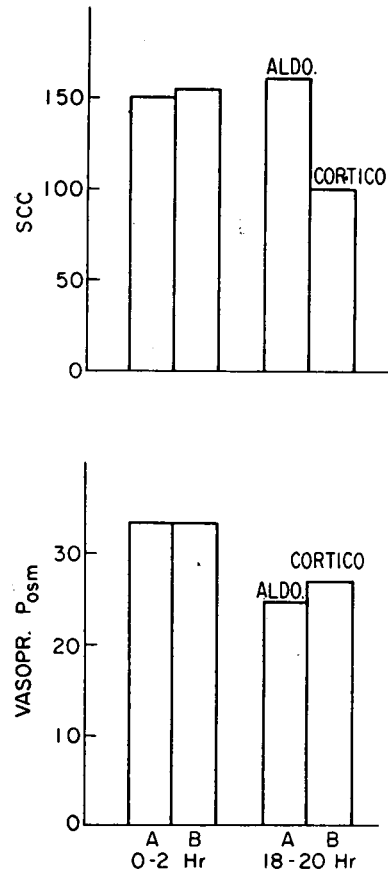

FIGURE 9 The effect of 2×10^{-9} M deoxycorticosterone (DOC) and 10^{-8} M corticosterone on the SCC of paired tissues and on the P_{osm} response to vasopressin of paired tissues. The mean ratio $\dfrac{\text{DOC } (18-20 \text{ hr})/(0-2 \text{ hr})}{\text{cortico } (18-20 \text{ hr})/(0-2 \text{ hr})}$ for SCC $= 1.10$ (n $= 10$), and for the P_{osm} response to vasopressin $= 0.90$ (n $= 13$, $P < 0.05$).

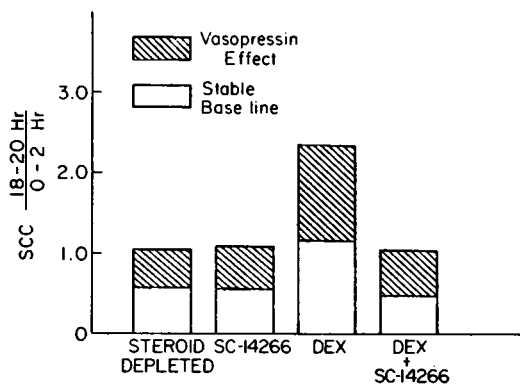828   J. S. Handler, A. S. Preston, and J. Orloff

FIGURE 11 The effect of steroid depletion, 5×10^{-6} M SC-14266, 3.5×10^{-9} M dexamethasone (Dex) and Dex $+ 5 \times 10^{-6}$ M SC-14266 on SCC and the SCC response to vasopressin in tissue from the same toad bladder, experiments performed in the spring. The mean base line SCC at 0–2 hr were 109, 104, 100, and 107, respectively. The ordinate is the ratio of the SCC at 18–20 hr to the SCC at 0–2 hr. The mean SCC (18–20 hr)/(0–2 hr) for (Dex + SC-14266)/Dex was 0.48 ($P < 0.005$) for base line and 0.48 ($P < 0.01$) for vasopressin effect (n = 6).

10^{-9} M dexamethasone, and 3.5×10^{-8} M dexamethasone vs. dexamethasone plus SC-14266 were performed. In these experiments, dexamethasone had the usual stimulatory effect on the P_{osm} response to vasopressin, SC-14266 had no effect by itself, and in contrast to the SCC experiments, had no significant effect on the dexamethasone enhancement of the P_{osm} response to vasopressin. In the second series (Fig. 13), the SCC and the SCC response to vasopressin, (18–20 hr)/(0–2 hr), were higher in all preparations than in the spring (Fig. 11). In addition, the effect of dexamethasone was greater and was only partially inhibited by the spirolactone. The behavior of the P_{osm} response to vasopressin was also different in the fall (Fig. 14). In these, the "steroid-depleted" tissue did not exhibit as marked a fall in the P_{osm} response to vasopressin as in the first series, and the effect of 3.5×10^{-9} M dexamethasone was not as great. In the second series, as in the first, there was a tendency for slight inhibition of the dexamethasone effect by SC-14266 (Dex + SC-14266/Dex). The additional experiments in Fig. 14 demonstrate that dexamethasone has an effect on the P_{osm} response to vasopressin in the presence of the spirolactone. Although the spirolactone inhibited the effect of dexamethasone on SCC and had no effect on the hormone's enhancement of the P_{osm} response to vasopressin in the first series, as would be expected were these two separate steroid hormone interactions with the bladder, the separation of the steroid's effects on the two systems was not as marked in the second series and these experiments cannot be considered conclusive evidence for two separate steroid hormone-tissue interactions.

The concept of mineralocorticoid and nonmineralocorticoid systems in the toad bladder was extended further by experiments in which the rate of oxidation of glucose-1-^{14}C and glucose-6-^{14}C was measured. Kirchberger, Martin, Leaf, and Sharp (15) have reported that in toad bladder, aldosterone decreases the difference in the rate of oxidation of glucose-1-^{14}C compared with the rate of oxidation of glucose-6-^{14}C [(^{14}C-1) − (^{14}C-6)] and stimulates the latter. Their observations were confirmed in experiments summarized in Fig. 15. Kirchberger et al. also reported that although the effect of aldosterone on the oxidation of glucose-6-^{14}C was eliminated when sodium was removed from the Ringer's solution bathing the mucosal surface of the bladder (thus eliminating sodium transport), the steroid still reduced the difference between the rate of oxidation of glucose-1-^{14}C and glucose-6-^{14}C. Experiments in Fig. 16 with dexamethasone and SC-14266 were performed to explore the possibility that the effect of adrenal steroid
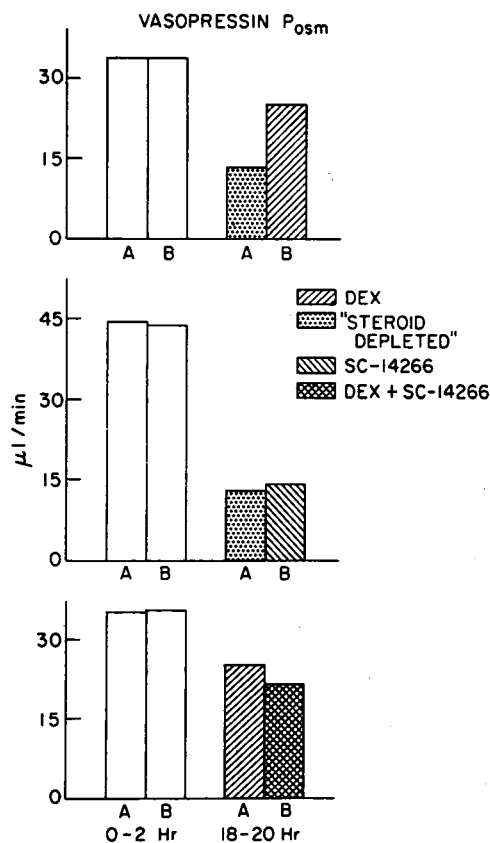

FIGURE 12 The effect of steroid depletion, 3.5×10^{-9} M Dex, 5×10^{-6} M SC-14266, and Dex + SC-14266 on the P_{osm} response to vasopressin in paired tissues, experiments performed in the spring. The mean P_{osm} responses to vasopressin (18–20 hr)/(0–2 hr) were Dex/Depleted = 1.95 ($P < 0.001$), n = 8, (SC-14266)/Depleted = 1.14, n = 9, and (Dex + SC-14266)/Dex = 0.86 ($P < 0.1$), n = 13.
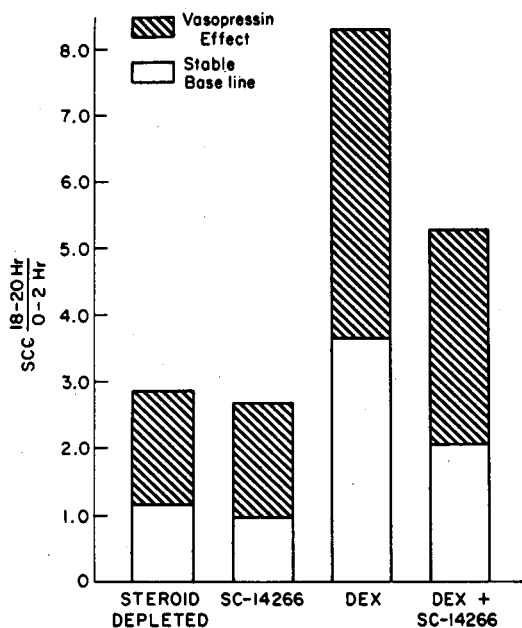
FIGURE 13 The effect of steroid depletion, 5×10^{-6} M SC-14266, 3.5×10^{-9} M Dex and Dex + SC-14266 on SCC and the SCC response to vasopressin in tissue from the same bladder, experiments performed in the fall. The mean base line SCC at 0–2 hr were 75, 62, 60, and 71, respectively. The mean SCC (18–20 hr)/(0–2 hr) for (Dex + SC-14266)/Dex was 0.56 ($P < 0.005$) for base line and 0.69 ($P < 0.05$) for vasopressin effect (n = 8).

hormones on glucose-6-^{14}C oxidation is mediated by the mineralocorticoid system, and that the effect on [(^{14}C-1) −(^{14}C-6)] glucose oxidation is mediated by a non-mineralocorticoid system. The first experiment demonstrates that 8×10^{-9}M dexamethasone has an effect on glucose oxidation similar to that of aldosterone.[2] The spirolactone alone had no effect on glucose oxidation, but when added to 8×10^{-9}M dexamethasone inhibited the effect of the hormone on glucose-6-^{14}C oxidation without altering the effect of the hormone on [(^{14}C-1)− (^{14}C-6)] glucose oxidation. If the assumption that the spirolactone inhibits primarily mineralocorticoid activity is valid, these results are compatible with the interpretation that the effect of dexamethasone on glucose-6-^{14}C oxidation which is blocked by SC-14266 is a mineralocorticoid effect of the hormone, and the effect of the hormone on [(^{14}C-1)−(^{14}C-6)] glucose oxidation which is not blocked by the spirolactone is not a mineralocorticoid effect of dexamethasone.

Finally, in view of the relationship of the electrolyte composition of the toad bladder to the P_{osm} response of the bladder to vasopressin (8), and reports indicating

[2] Lower concentrations of dexamethasone as used in Figs. 11 and 12 did not have statistically significant effects on the oxidation of glucose-1-^{14}C and glucose-6-^{14}C.

that aldosterone in vitro can alter the electrolyte composition of tissues (16, 17), the effect of aldosterone on the electrolyte composition of the bladder was studied. The data in Table I indicate that under conditions in which aldosterone elicits a marked stimulation of transbladder sodium transport (SCC) and the P_{osm} response to vasopressin compared to steroid-depleted tissue, the hormone has no detectable effect on cell water or electrolyte content. In addition, the electrolyte and water composition of tissue depleted of steroid effect (18 hr in vitro) was not different than that of fresh tissue sampled within an hour of removal from the toad. There was also no significant difference in the cell electrolyte composition of bladders removed from toads kept in 0.6% NaCl, which presumably suppresses aldosterone secretion (5), and of bladders from toads kept on bedding moistened with tap water. It is likely that the latter group of bladders exhibited a greater endogenous aldosterone effect in that on a dry weight basis the stable SCC (18.6 μamp/mg) within 2 hr of removal from the toad was considerably higher ($P < 0.02$) than the SCC (11.2 μamp/mg) of bladders removed from toads kept in 0.6% NaCl. These effects of aldosterone and of 0.6% NaCl are similar to those found by Crabbé who reported SCC/area and tissue electrolyte composition without correction for extracellular space (5). These data must be interpreted with caution. The toad bladder has a relatively large inulin space ($\sim 50\%$ of total tissue water) which hinders accurate estimation of cell sodium. There is also evidence of "ion binding" by the tissue since the concentration of tissue electrolytes exceeds the concentration in the Ringer's solution (5, 18, 19, and Table I, Time₀). Thus, although there are no significant changes in Table I or in the study by Crabbé (5), it is still conceivable that aldosterone causes a critical but thus far undetected change in the electrolyte composition of certain epithelial cells in the bladder.

DISCUSSION

The effects of adrenal steroid hormones on the permeability characteristics of the toad bladder and the relationship between the effects of adrenal steroid hormones and neurohypophyseal hormones have been examined in several laboratories. A number of observations in this study differ from those reported previously by others. Marumo (20), using Bufo bufo japonicus, reported that cortisol added to urinary bladders depleted of endogenous steroid hormone caused a fall in P_{osm} and P_{H_2O} but had no effect on P_{urea}, SCC, or the P_{osm}, P_{H_2O}, or SCC response to vasopressin. The differences between the observations of Marumo and those in Figs. 6 and 7 of this study (with aldosterone) may be due to the brief exposure to steroid hormone in his studies (2 hr vs. 18 in the present studies) and to species differ-
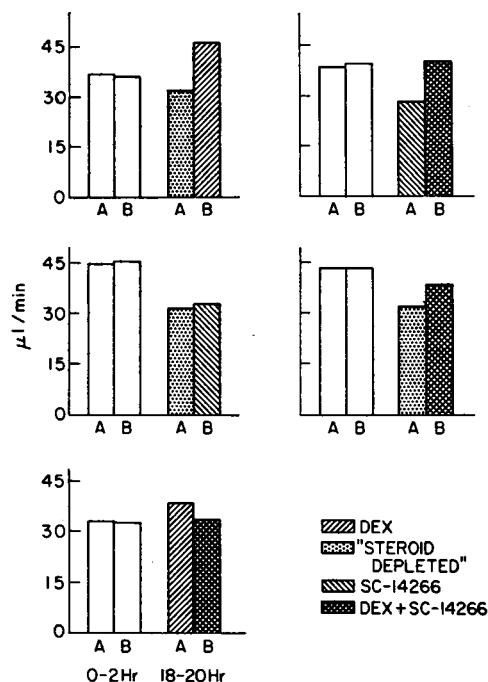
FIGURE 14 The effect of steroid depletion, 3.5×10^{-9} M DEX, 5×10^{-6} M SC-14266, and Dex + SC-14266 on the P_{osm} response to vasopressin in paired tissues, experiments performed in the fall. The mean P_{osm} responses to vasopressin (18–20 hr)/(2–0 hr) were

Dex/Depleted = 1.47 ($P < 0.005$), n = 6,
(SC-14266)/Depleted = 1.02, n = 6,
(Dex + SC-14266)/Dex = 0.87 ($P < 0.01$), n = 10,
(Dex + SC-14266)/Depleted = 1.21 ($P < 0.001$), n = 10,

and

(Dex + SC-14266)/SC-14266 = 1.43 ($P < 0.01$), n = 6.

ences. Crabbé (21) found that incubation for 4 hr with aldosterone, cortisol, or corticosterone had no effect on P_{osm} or P_{H_2O} or the increase in P_{osm} and P_{H_2O} in response
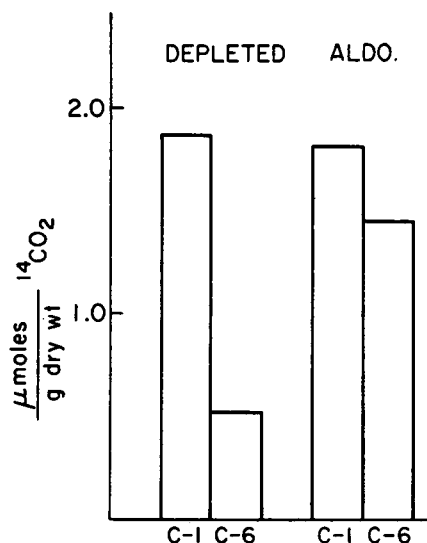

FIGURE 15 The effect of steroid depletion and of 2×10^{-7} M aldosterone on the oxidation of glucose-1-^{14}C and glucose-6-^{14}C. The differences (Aldosterone − Depleted) are, C-6 = + 0.93 ±0.15 ($P < 0.001$), and (C-1) − (C-6) = − 0.99 ±0.24 ($P < 0.005$), n = 8.

to vasopressin. He also did not find the enhancement by aldosterone of the SCC response to vasopressin that has been described by Fanestil et al. (9) and confirmed in this report. In Crabbé's experiments, the vasopressin response of control and steroid-treated bladders was examined several hours after the bladder was removed from toads which had been maintained in distilled water. It is probable that under these conditions the control bladders still had a considerable steroid effect from endogenous adrenal steroids obscuring the effects of exogenous hormones. Porter and Edelman (22) have demonstrated that prolonged incubation in vitro increases the sensitivity of the toad bladder to adrenal steroid hormones.

TABLE I

Effect of Aldosterone and of Steroid Depletion on the Cell Water Content and the Concentration
of Na, K, and Cl in Cell Water

		Cell H₂O	Cell electrolytes		
			Na	K	Cl
		ml/mg dry wt.		μEq/ml	
Time₀ tap water*	(10)	2.40 ±0.06	81.2 ±2.4	124 ±3.2	78.4 ±1.9
0.6% NaCl‡	(11)	2.37 ±0.12	93.1 ±5.2	123 ±4.0	78.7 ±1.7
Δ(depleted − time₀)§	(8)	+ 0.01 ±0.10	− 18.0 ±9.6	+ 3.0 ±4.2	− 3.2 ±2.5
Δ(aldo − depleted)‖	(7)	− 0.12 ±0.07	+ 6.8 ±5.9	+ 1.0 ±3.5	+ 3.1 ±2.8

*Bladders removed from toads kept in tap water.
‡ Bladders removed from toads kept in 0.6% NaCl for 2–5 days. These were also used in the experiments testing the effect of aldosterone and of steroid depletion.
§ Steroid-depleted tissue (18–20 hr) minus paired samples from the same tissue at 0–2 hr.
‖ Tissue incubated with 2×10^{-7} M aldosterone for 18–20 hr minus paired steroid-depleted tissue at 18–20 hr.

Effect of Adrenal Steroid Hormones on Response of Toad Bladder to ADH 831
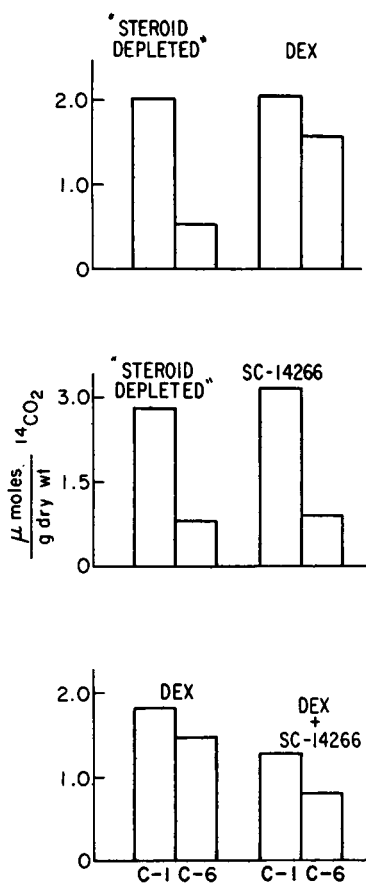
FIGURE 16 The effect of steroid depletion, 8×10^{-9} M Dex, 5×10^{-6} M SC-14266, and Dex + SC-14266 on the oxidation of glucose-1-^{14}C and glucose-6-^{14}C in paired tissue. The differences are: Dex − Depleted, C-6 = + 1.02 ±0.15 ($P < 0.01$), and (C-1) − (C-6) = − 0.98 ±0.22 ($P < 0.001$), n = 12; SC-14266 − Depleted, C-6 = + 0.22 ±0.19, and (C-1) − (C-6) = + 0.46 ±0.43, n = 8; Dex + SC-14266 − Dex, C-6 = − 0.68 ±0.16 ($P < 0.005$), and (C-1) − (C-6) = + 0.11 ±0.24, n = 11.

Sharp and Leaf (3) also did not find enhancement by aldosterone of the SCC response to vasopressin in fresh tissue.

The experiments in Figs. 8–10 demonstrating greater relative activity of aldosterone and DOC, two mineralocorticoid hormones, on SCC than on the P_{osm} response to vasopressin and the opposite pattern for dexamethasone and corticosterone, two glucocorticoid hormones, are compatible with the suggestion that the two effects of adrenal steroid hormones are mediated by separate systems with differing affinities for mineralocorticoid and glucocorticoid hormones. The difference in activity of each steroid on the two systems was considerably less than is found in more classical assay systems for these hormone types (23). The relatively small difference in activity may represent the true difference in affinity of the two systems in this tissue, may be the result of suboptimal assay conditions, or may be explained by a more complex model than proposed here. In any event, the simplest explanation, i.e., two separate hormone-tissue interactions, is supported further by the experiments with the spirolactone SC-14266. This agent, which is generally thought to inhibit competitively the binding of adrenal steroid hormones at mineralocorticoid receptor sites (14, 24, 25), markedly inhibited the effect of dexamethasone on SCC and the SCC response to vasopressin (Figs. 11 and 13), and had only a slight inhibitory effect on the dexamethasone-induced enhancement of the P_{osm} response to vasopressin. Experiments have not been performed to determine whether the enhancement of the P_{urea} response to vasopressin is a mineralocorticoid or nonmineralocorticoid effect. The extension of the idea of two hormone-tissue interactions to the observation that SC-14266 inhibits the stimulation by dexamethasone of glucose-6-^{14}C oxidation without altering the dexamethasone-induced reduction in the rate of oxidation of [(^{14}C-1)−(^{14}C-6)] glucose (Fig. 14) would indicate that the reduction [(^{14}C-1)−(^{14}C-6)] glucose oxidation (presumably hexose monophosphate shunt activity) by aldosterone in both sodium-containing and sodium-free media (15) can be explained as an effect on the glucocorticoid system rather than a mineralocorticoid effect that is independent of sodium transport.

Although there is a considerable amount of evidence indicating the important role of protein synthesis in the response of the toad bladder and mammalian kidney to aldosterone (2), the nature of the ultimate changes in the tissue that are responsible for increased rates of sodium transport are in dispute (2, 3). Experiments in Figs. 2 and 4, in which aldosterone is shown to enhance the SCC and P_{osm} response to cyclic AMP, indicate that the enhancement by aldosterone of the response to vasopressin probably occurs after the adenyl cyclase step in which the production of cyclic AMP is stimulated by vasopressin. In studying another cyclic AMP-mediated hormone response, the stimulation by glucagon of gluconeogenesis from lactate in the perfused rat liver, Friedmann, Exton, and Park (26) have found that in livers taken from adrenalectomized rats, dexamethasone enhances the response to glucagon and to cyclic AMP. They suggested that the glucocorticoid effect in the perfused rat liver is at a step after the producton of cyclc AMP.

Finally, it is appropriate to note the abnormally low free water reabsorption by the kidneys of adrenalectomized animals and its correction by glucocorticoid hormones. Although this effect of glucocorticoid hormones has been attributed to enhancement of sodium reabsorption by the ascending limb of the loop of Henle (27), the restoration of normal free water reabsorption by glucocorticoid hor-

mones could be due at least in part to an effect on the permeability response of the distal nephron to vasopressin, an effect analogous to that seen in this study with the toad bladder.

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