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Effect of Hormonal Status on Renal Ornithine Decarboxylase Activity (41162)

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Abstract. In mice deprived of water, renal ornithine decarboxylase (ODC) activity declined rapidly and remained depressed for up to 3 days. Dehydration, via substitution of 2% NaCl for drinking water, produced an initial decline in kidney enzyme activity followed by a continuing increase throughout a 4-day treatment period. Arginine vasopressin (AVP) injection caused a rapid, transient elevation in ODC activity in the kidney. Adrenalectomy had no effect on basal ODC activity in the kidney, whereas propylthiouracil treatment and gonadectomy (acute) caused a marked reduction in enzyme activity. The renal ODC response to AVP injection was blunted in each of these endocrinologically altered mice. Basal ODC levels were normal in mice 120 days postorchidectomy, as was the response to AVP. These results indicate that some of the effects of dehydration on renal ODC may be due to mechanisms other than those induced by the normal secretory products of the posterior pituitary.

The polyamines are aliphatic nonprotein bases present as normal cellular components in almost all living species (1). Much attention has been focused recently on the role of polyamines in the growth and differentiation of normal and neoplastic tissues (2), but no definitive statements can yet be made concerning their function(s). Ornithine decarboxylase (ODC, EC 4.1.1.17) is the rate-limiting enzyme in the biosynthetic pathway leading from ornithine to putrescine, the simplest of the polyamines. ODC activity is regulated in part by hormones of the endocrine system, with both steroids and peptides affecting ODC activity in various organs (3–6).

Dehydration is a potent stimulus for the increased synthesis and release of the posterior pituitary hormones (7, 8). After 3 days of water deprivation the pituitary content of antidiuretic hormone (ADH) falls to less than half the normal value (9, 10). In addition to its effect on hormones of the neurohypophysis, water deprivation inhibits the compensatory ovarian hypertrophy normally seen after unilateral ovariectomy (11), increases serum prolactin levels (12), and alters the circadian rhythm of pituitary–adrenal activity (13). Salt loading, as a means of dehydration, activates the neurohypophysis in a similar manner causing increased secretion of ADH (14). Acute hemorrhage (15) and hypothyroidism (16, 17) also lead to increased plasma levels of vasopressin. In each of these pathophysiologic conditions the kidney is a major target organ for the elevated plasma vasopressin.

Among the many effects of vasopressin on the kidney is stimulation of ODC activity (18). In a previous study (19) we observed that acute injections of arginine vasopressin (AVP) increased renal ODC activity. However, with chronic elevation of plasma AVP (resulting from dehydration, salt loading, or multiple injections of AVP), the renal ODC activity was depressed to below normal levels. The purpose of the experiments reported here was to define further this bimodal response of renal ODC and to examine the interplay of other hormones which affect kidney function.

Materials and Methods. Experiments were performed on adult male mice of the Nya:NYLAR strain weighing 20–30 g. The animals were kept in quarters controlled for temperature (22°) and light (0700–2100, lights on). Except as noted, food pellets and water were given *ad libitum*. Surgical procedures were performed under ether anesthesia. Controls for the chronically gonadectomized mice were matched for age and received sham-operative procedures

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simultaneously with the experimental group.

AVP and lysine vasopressin were diluted in 0.9% saline and given intraperitoneally as a single injection of 3 IU/100 g of body wt. Propylthiouracil (PTU) was administered in the drinking water as a 0.5% solution. Mice were maintained on the PTU treatment for 8 weeks before being used experimentally. Animals matched for age and receiving normal drinking water were used as controls for the thyroid experiments. Serum T_4 levels were measured by radioimmunoassay (Clinical Assays).

Renal ODC activity was estimated from the *in vitro* liberation of $^{14}CO_2$ (19). Reaction vials contained 0.3 m*M* pyridoxal phosphate, 5 m*M* dithiothreitol, and 3 nmol of L-ornithine, including 0.05 μCi of DL-[1-^{14}C]ornithine in a final volume of 100 μl. All values for each experiment were determined in duplicate, in the same assay. To avoid a reported diurnal variation in renal ODC activity (20), all mice were killed before noon. For procedures in which the samples could not be assayed the same day as collected, the homogenized specimens were kept frozen at −20° and assayed the following day. No change in activity could be detected as a result of storage. DL-[1-^{14}C]Ornithine monohydrochloride was obtained from New England Nuclear. Other reagents were supplied by Sigma Chemical Company.

The data were analyzed by Student's *t* test. Results were considered significant at $P < 0.05$.

Results. Water deprivation produced a marked decrease in renal ODC activity within 2 hr. After 12 hr of deprivation the activity had plateaued at the lowest levels (8% of control); it remained there for the 3-day observation period (Table I).

Substitution of 2% NaCl for drinking water produced an initial decline in renal ODC activity (12 hr), followed by an increase which continued through the 4 days of observation (Table I).

When AVP was given acutely, maximum renal ODC activity occurred at 2-hr postinjection (Fig. 1). At 4 hr it was still elevated fivefold above control, but by 8 hr it had returned to basal levels. A similar response profile was seen with lysine vasopressin, although the maximum response was smaller (18 vs 60 pmol $^{14}CO_2 \cdot hr^{-1} \cdot mg^{-1}$) for equal amounts.

TABLE I. EFFECTS OF WATER DEPRIVATION OR SALT LOADING ON RENAL ODC ACTIVITY IN MICE

Treatment and time (Hr.)	ODC activity (mean ± SEM)[a]	Percentage of normal
Water deprivation		
0	11.83 ± 3.11	100
2	7.48 ± 1.25[b]	63
4	5.89 ± 2.28[b]	50
8	4.65 ± 0.74[b]	39
12	1.81 ± 0.21[b]	15
24	1.61 ± 0.22[b]	14
48	1.76 ± 0.21[b]	15
60	1.10 ± 0.12[b]	9
72	0.84 ± 0.39[b]	7
Salt (2% NaCl)		
0	2.45 ± 0.22	100
12	0.86 ± 0.04[b]	35
24	1.42 ± 0.18[b]	58
48	5.48 ± 0.09[b]	224
72	4.47 ± 0.41[b]	182
96	9.56 ± 1.21[b]	390

[a] Measured as pmole $^{14}CO_2$/hr/mg tissue; (N = 8–12 mice for each time period).
[b] $P < 0.05$ vs control.

The effects of removal of various organs on renal ODC are shown in Table II. Basal levels of enzyme activity exhibit some variability from one experiment to another (18, 21, 22). Therefore, concurrent controls were utilized for each treatment group. Ad-

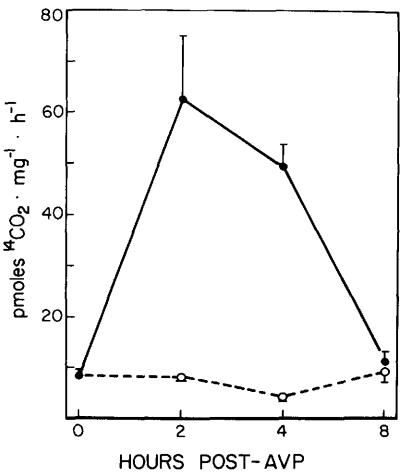

FIG. 1. Renal ODC in mice after ip injection of AVP (3 IU/100 g body wt). Dashed line represents vehicle-injected mice. Vertical bars show the SEM (N = 18).

TABLE II. EFFECT OF ORGANECTOMY AND ARGININE VASOPRESSIN ON RENAL ODC ACTIVITY

Organ and time after operation	ODC activity[a]			
			AVP (3 IU/100 g)	
	Control	Organectomy	Control	Organectomy
Adrenal				
5 Days	5.36 ± 0.36	6.38 ± 0.55[c]	29.63 ± 2.68[d]	21.31 ± 2.31[d,e]
Thyroid				
8 Weeks[b]	16.24 ± 2.12	7.72 ± 0.72[d]	57.45 ± 7.72[d]	35.23 ± 5.02[d,e]
Gonads				
10 Days	5.53 ± 0.86	2.46 ± 0.27[d]	29.60 ± 2.44[d]	7.25 ± 1.17[e,e]
120 Days	6.73 ± 1.14	6.76 ± 0.50[c]	30.03 ± 3.61[d]	25.83 ± 3.05[d,f]

Note. Values are mean ± SEM (N = 8–12 mice). Controls for thyroid and gonad experiments were age-matched with treatment group.

[a] Measured as pmole $^{14}CO_2$/hr/mg tissue.

[b] Eight weeks of PTU treatment (0.5% solution as drinking water).

[c] Not significant vs control.

[d] $P < 0.01$ vs control.

[e] $P < 0.05$ vs AVP control.

[f] NS vs AVP control.

renalectomized mice showed no change in renal ODC at postsurgical Day 5. AVP injection in these adrenalectomized animals produced an increase in enzyme activity equal to 73% of that in control animals.

Treatment with PTU reduced thyroxine to less than 1 μg/dl (control, 6 μg/dl) and reduced renal ODC activity by up to 50% compared to control animals. The response of the PTU-treated mice to AVP injections was lower (61%) than that of the control animals.

Gonadectomy (acute) induced a marked decline in renal ODC activity 10 days after surgery. At that time the surgically altered mice also showed a blunted response to AVP injection (25%) when compared to sham controls. By 120 days postgonadectomy the basal ODC activity in the kidney had returned to control levels, and the response to AVP injection was similar to that of sham-injected mice.

Discussion. In an earlier report we demonstrated that one action of ADH in the kidney is to increase ODC activity (19). However, this increased activity was seen only after an acute treatment with AVP, whereas chronic administration of AVP over a 3-day period caused a decline in renal ODC similar to that of dehydration. This decline was apparently an effect of the chronic dosage form, rather than the quantity of AVP, since equivalent doses of AVP given acutely produced an increase in renal ODC. A similar biphasic response in kidney ODC after cortisone injections has been reported by Andersson, *et al.* (23). These results, together with the rapid response to AVP injection (2 hr post-treatment), suggest that the role of ODC in the kidney may be more than that of a regulator of protein synthesis (24) and could, in fact, involve the energy metabolism or transport processes of this very active organ. Experiments are in progress to test this hypothesis.

In the dehydrated animal with high concentrations of endogenous ADH, there was a marked and persistent decline in renal ODC activity, which was significant after only 2 hr of water deprivation. Despite continued stimulation by endogenous ADH the enzyme levels did not return toward baseline. This is in contrast to other organs, such as the pituitary (25) and thyroid (26), where the ODC activity returns to baseline within hours or days of the hormonal stimulus. This could be due to a basic difference in end-organ physiology or to a change in organ responsiveness to the

stimulus. However, if the kidney does become unresponsive to ADH stimulation (16), one would expect the ODC activity to return toward normal, as reported for the pituitary (25). Alternatively, other factors (hormones) resulting from the stress of dehydration may be modifying the effects of the elevated ADH levels.

When the animals were dehydrated by salt loading, the changes in renal ODC were quite different. After a small initial decline the ODC activity increased steadily to well above baseline, even though ADH levels remained elevated, as in water deprivation (10, 14). This difference could be due to the increased daily fluid consumption of the salt-loaded mouse (28 vs 14 ml in controls after 60 hr). This doubled fluid load would be a strong stimulus to increased renal activity. Alterations in mineralocorticoid activity at the nephron due to the increased salt intake may also be a factor in the steadily rising enzyme activity. Involvement of the adrenal hormones is questionable; however, since—as shown here and as reported by Andersson, *et al.* (23)—adrenalectomy has no effect on basal kidney ODC activity.

Hypothyroidism is associated with several disorders of renal function. Hypothyroid rats excrete more urine (27), have less urinary concentrating ability (28), and show an impaired diuresis following a water load (29). Seif *et al.* (16) found plasma vasopressin elevated in hypothyroid rats and suggested that factors other than vasopressin may be more important in the renal handling of salt and water in hypothyroidism. Our results seem to confirm this assertion, since the decline in renal ODC activity in the hypothyroid mice was only about 50% of control and did not approach the very low levels in dehydrated animals. AVP injection increased kidney ODC activity in the hypothyroid mice but not to the extent seen in control mice. Acute injection of T_4 into the PTU-treated mice caused a return toward control values in renal enzyme activity (Pass and Vallet, unpublished observations). One of the effects of PTU treatment is to stimulate the synthesis and release of thyroid-stimulating hormone via thyrotropin-releasing factor (TRF). Since TRF can either stimulate (30) or inhibit (31) the release of ADH, the role of this neuropeptide in the present experiments cannot be accurately defined. Nevertheless, it seems likely that part of the dehydration-induced decline in renal ODC may be attributable to depressed thyroid function.

The decline in renal ODC noted 10 days after castration appears to be transient. In long-term castrates (120 days) renal ODC activity had returned to normal, and the response to AVP was similar to that in intact animals. Since water deprivation alters gonadal function, possibly through a direct effect on the gonads (11), it is reasonable to assume that the stimulatory actions of testosterone on kidney ODC (32) may have been blunted in the dehydrated animals, thereby contributing to the prolonged low enzyme activity.

In summary, dehydration is an easily controlled, well-defined stress which produces a myriad of effects on the animal, with the kidney as the ultimate target organ for many of these processes. The present experiments demonstrate that alterations in adrenal or gonadal (chronic) function do not change the basal or AVP-stimulated kidney ODC activity. It appears that thyroid gland secretions or other peptide hormones may exert some tonic control over kidney function (as measured by changes in ODC activity), and could account for some of the changes seen during dehydration. We are continuing to investigate these possibilities.

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