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The Role of the Adrenal Gland in Control of H^+ and NH_4^+ Excretion by Toad Urinary Bladder[1] (41593)

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Abstract. The urinary bladder of *Bufo marinus* has been shown to excrete H^+ and NH_4^+ and this excretion is increased by metabolic acidosis. The involvement of the adrenal gland and its steroid secretions in the adaptation for increased acid and ammonia excretion by the bladder was tested during the course of this study. Groups of toads were adrenalectomized and maintained in chronic NH_4Cl-induced acidosis. Three other groups of toads were adrenalectomized and put in acidosis but repleted with 2.5 mg/day of either cortisol (CT), dexamethasone (Dexa), or deoxycorticosterone acetate (DOCA). All control groups were sham-operated. The bladders were excised after 3 days and mounted between 2-ml Lucite chambers. Net H^+ and NH_4^+ fluxes into the mucosal media were measured and reported in units of nanomoles per 100 mg bladder per minute. In control acidotic toads H^+ excretion was 20.1 ± 2.0 and the adrenelectomized nonreplete group H^+ excretion was 14.2 ± 1.87 ($P < 0.04$). For the same groups NH_4^+ excretion was 2.90 ± 0.26 for the controls and 1.38 ± 0.19 for the adrenalectomized ($P < 0.001$). The H^+ excretion in CT-, Dexa-, and DOCA-repleted toads was not significantly different from the control group. NH_4^+ excretion, however, showed a 55% decrease ($P < 0.001$) in the CT group, and a 45% decrease ($P < 0.05$) in the Dexa group. The NH_4^+ excretion in the DOCA repleted group was significantly different from the control group. Therefore, we conclude that the adrenal gland plays a role in the adaptive increase of H^+ and NH_4^+ excretion by the urinary bladder in acidosis through the secretion of steroid hormones. The increase in NH_4^+ excretion appears to be a mineralocorticoid-stimulated process. We were not able to determine in this study if the steroid hormones had an exacting regulatory role or one of a permissive role over H^+ and NH_4^+ excretion in the toad urinary bladder.

The urinary bladder of *Bufo marinus* has been shown to excrete H^+ and NH_4^+ and this excretion increases in metabolic acidosis (1). Several laboratories have worked to determine the relationship of the adrenal gland and its secretions, specifically aldosterone, to acidification and ammonia excretion. Ludens and Fanestil (2) and Frazier and Zachariah (3) have both shown a positive correlation between aldosterone and an increase in both acidification and ammonia excretion in the toad urinary bladder. Al-Awqati (4) reported similar effects of aldosterone on the acidification mechanism in the turtle bladder. Welbourne (5) demonstrated in rats that the administration of aldosterone results in increased ammonia excretion and metabolic alkalosis. Tannen and Ross (6) implicated aldosterone in the control of acid excretion in perfused rat kidneys. However, Tereo and Tannen (7) were unable to demonstrate this effect in similarly perfused rat kidneys.

The present study was designed to test the hypothesis that the adrenal gland may be important in the control of overall acid–base balance and, if so to further determine which adrenal steroid might be involved in this mechanism.

Materials and Methods. The toads used in these experiments were *Bufo marinus* of Mexican origin purchased from Wm. A Lemberger, Ltd. of Germantown, Wisconsin. The solutions, the procedure of inducing acidosis, the procedure for inducing surgical anesthesia, the procedure of measuring H^+ and NH_4^+ excretion, and the procedure for obtaining plasma were as previously described (1, 3, 8, 9). Adrenal glands were removed by cauterization using Coles Radiosurg Electronic Scalpel IV. Control toads were sham-operated by opening the body cavity in the same manner as adrenalectomies but no cauterization was performed. Verification of removal of the adrenal gland by cauterization

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was made in all toads by radioimmunoassay (RIA) of plasma aldosterone levels and routine staining (H&E) and sectioning of kidneys. The RIA procedure used was a ^{125}I-aldosterone test kit purchased from International CIS through Damon Diagnostics of Needham Heights, Massachusetts.

The basic procedure was to treat the animals by one of the protocols given below. Following this treatment the animals were sacrificed and the urinary bladder removed. The bladders were mounted as sheets between 2-ml Lucite chambers. Both serosal and mucosal chambers contained 2 ml of a Ringer solution which contained in m*M*: NaCl, 114; KCl 3.0; CaCl, 0.9; sodium phosphate buffer 1.5; the final pH was 7.0. After mounting, the bladders were allowed to equilibrate 15–30 min. The excretion of H^+ and NH_4^+ was then measured for a 2-hr period. At the end of this period values for pH and NH_4^+ were determined in both muscosal and serosal solutions. Excretion of H^+ was calculated from the change in pH of the mucosal fluid and the amount of NH_4^+ in the mucosal fluid was determined colorimetrically (10). These values were then normalized for bladder wet weight and time and reported in units of nanomoles per 100 mg bladder per minute. The groups were compared statistically using Welch's approximation to the *t* test (11) to determine significance. Plasma K^+ concentrations were determined on an IL digital flame photometer.

Five experimental protocols were utilized as follows:

1. Basal acidification rate. Four toads were sacrificed and bladders mounted to determine basal acidification rate, and NH_4^+ excretion rate, and aldosterone levels of normal, non-acidotic toads.

2. Acidotic acidification rate. Four toads were put into acidosis and maintained for 3 days. On the third day they were sacrificed and excretion rates determined to verify that acidification rate increased under acidotic conditions.

3. Adrenalectomized (Adx), acidotic toads. Sixteen toads were kept in Adx state in 120 m*M* NaCl solution for 4 days. Sixteen toads were sham-operated for use as controls and kept in tap water. Acidosis was induced as previously described. On the fourth day all toads were sacrificed and excretion rates determined.

4. Adx, cortisol-replete, acidotic toads. Eleven Adx toads were kept acidotic in normal tap water for 4 days. On the day of surgery each toad was given a 2.5-mg dose of cortisone acetate (CT) (T.M. Cortone acetate, Merck, Sharp and Dohme) by injection into the dorsal lymph sac. The dose on the days following until sacrifice was divided into equal halves and half of the 2.5-mg dose was administered twice each day. Nine toads were sham-operated and kept in tap water as controls. They did not receive injection of cortisol vehicle.

5. Adx, deoxycorticosteroid-, and dexamethasone-replete, acidotic toads. Twenty-five toads were Adx and kept acidotic in normal tap water for 4 days. They were divided into two groups following surgery. Fourteen of the toads were repleted with 2.5-mg doses of deoxycorticosterone acetate in 20% ethanol Ringers (DOCA). The other eleven were repleted with 2.5-mg doses of dexamethasone in 20% ethanol Ringers (Dex). The doses were administered as described for CT repletion. DOCA and Dex were purchased from Sigma Chemical Co., St. Louis, Missouri. The nine control toads were injected with 20% ethanol Ringers. The dose of steroid used in all of the above experiments was calculated from the dose used to achieve maximal response in an *in vitro* preparation (3). Therefore, the dosage used may approximate a pharmacologic level rather than a physiological level in the repletion studies.

Results. Results of the RIA for plasma aldosterone are shown in Table I. Cauterization removal of the adrenal resulted in a significant reduction in plasma aldosterone in all groups of toads tested. Blood samples were not taken from cortisol-replete toads. Aldosterone levels dropped from 0.14 ± 0.04 μg/100 ml for control levels to 0.03 ± 0.01 μg/100 ml for Adx, nonreplete toads and Adx, Dexa-replete toads and to 0.04 ± 0.01 for Adx, DOCA-replete toads.

Excretion rates for Adx, non-replete toads are shown in Table II. Removal of the adrenal resulted in a significant reduction in the excretion of both H^+ ($P < 0.04$) and NH_4^+ ($P < 0.001$). The H^+ excretion rate was reduced

TABLE I. PLASMA ALDOSTERONE LEVELS ON CONTROL AND ADRENALECTOMIZED TOADS

State of toad[a]	N	Aldosterone (μg/100 ml ± SEM)[b]	P value[c]
Control			
Normal, nonacidotic	4	0.10 ± 0.02	
Sham-operated, nonreplete	4	0.14 ± 0.04	
Sham-operated, ETOH-replete	8	0.18 ± 0.02	
Adrenalectomized			
Nonreplete	6	0.03 ± 0.01	<0.013
Dexa-replete	3	0.03 ± 0.01	<0.001
DOCA-replete	7	0.04 ± 0.01	<0.001

[a] All toads are in acidosis unless otherwise indicated.
[b] Values expressed are means calculated from values determined in four separate RIAs.
[c] P is the probability that the value is different from the control value (0.14 ± 0.04 or 0.18 ± 0.02, respectively).

by 30% and the NH_4^+ excretion rate was reduced by 52% compared to sham-operated control excretion rates.

The results of repletion studies and studies of Table II are summarized in Table III. Adx toads repleted with CT or Dex showed no difference in excretion rates of NH_4^+ compared to Adx, nonreplete animals. In comparison to control toads, CT-replete still showed a 55% reduction ($P < 0.001$) and Dexa-replete toads showed a 45% reduction ($P < 0.05$) in NH_4^+ excretion. However, the NH_4^+ rate in DOCA-replete toads is significantly increased by 260% ($P < 0.05$) over the Adx, nonreplete rate while showing no significant difference from the control rate ($P > 0.20$).

Although the H^+ excretion rate significantly decreased in Adx, nonreplete animals compared to the controls, no significant difference could be demonstrated between controls and any of the Adx-replete toads. Table III demonstrates that although the H^+ ion excretion rate in CT and Dexa-replete toads is very similar and essentially no different from Adx, nonreplete animals, significant ($P < 0.30$) was not reached, probably because of the small sample size. Further testing needs to be done before final conclusions are drawn with regard to H^+ excretion.

Excretion rates were determined on six normal and six Adx toads that were in normal acid–base balance. The excretion in units of nanomoles per 100 mg bladder per minute was $H^+ = 12.1 \pm 1.07$ and $NH_4^+ = 3.47 \pm 1.15$ for normal toads, and $H^+ = 7.42 \pm 1.52$ and $NH_4^+ = 1.78 \pm 0.34$ for Adx toads. This indicates that the adrenal gland is important for maintenance of H^+ excretion but its absence did not alter significantly NH_4^+ excretion in the urinary bladder of the toad, during normal acid–base balance ($P < 0.05$, $P > 0.20$, respectively).

Discussion. Adrenalectomy resulted in decreased plasma levels of aldosterone ($P < 0.01$) and decreased excretion of both H^+ ($P < 0.04$) and NH_4^+ ($P < 0.001$). Table I indicates that the cauterization procedure used in this study was effective in the removal of adrenal tissue. Although the presence of any aldosterone remaining in the plasma 4 days after Adx would indicate that a few viable adrenal cells probably still exist, the circulating levels are far below the levels for normal toads that we recorded, therefore we are referring to these groups as Adx. Also, these levels are much lower than the normal values reported earlier by Frazier and Zachariah (3).

The normal toad plasma aldosterone level was not different from the acidotic toad (0.10 ± 0.02 vs 0.14 ± 0.04 μg/100 ml), $P > 0.50$, in Table I. This is surprising since one would

TABLE II. EXCRETION RATES FOR CONTROL AND ADRENALECTOMIZED TOADS DURING ACIDOSIS

	N	H^+ Excretion rate[a] nmole (100 mg)$^{-1}$ min^{-1}	P value[b]	NH_4^+ Excretion rate[a] nmole (100 mg)$^{-1}$ min^{-1}	P value[b]
Control	25	20.12 ± 2.0	<0.04	2.90 ± 0.26	<0.001
Adx	9	14.21 ± 1.87		1.38 ± 0.19	

[a] Mean ± SEM.
[b] P is the probability that the value is different from the control value.

TABLE III. SUMMARY OF EXCRETION RATES FOR CONTROL AND COMPLETE ADRENALECTOMIZED TOADS DURING ACIDOSIS

Group and state of toads	N	H^+ Excretion[a] nmole (100 mg)$^{-1}$ min^{-1}	NH_4^+ Excretion[a] nmole (100 mg)$^{-1}$ min^{-1}
(A) Sham-operated control	25	20.12 ± 2.0	2.90 ± 0.26
(B) EtOH-replete control	9	21.72 ± 2.4	2.24 ± 0.30
(C) Adx, nonreplete	9	14.21 ± 1.87	1.38 ± 0.19
(D) Adx, CT-repelete	5	14.70 ± 3.9	1.30 ± 0.28
(E) Adx, Dexa-replete	5	14.94 ± 4.0	1.23 ± 0.30
(F) Adx, DOCA-repelete	9	25.42 ± 2.14	3.59 ± 0.94

	Comparison probabilities	
Groups	P value	P value
A:B	<0.62 NS[b]	<0.12 NS
C:A	<0.04	<0.001
D:A	<0.30 NS	<0.001
E:B	<0.20 NS	<0.05
F:B	<0.30 NS	<0.20 NS
D:C	<0.90 NS	<0.80 NS
E:C	<0.90 NS	<0.70 NS
F:C	<0.001	<0.05

[a] Mean ± SEM.
[b] NS indicates $P > 0.05$.

naturally expect the plasma levels to increase in acidosis if aldosterone is involved in this adaptative increase of H^+ and NH_4^+ excretion by the bladder. However, a previous study by Frazier and Zachariah (3) reported that the plasma aldosterone levels did not change. Their findings are consistant with what we have reported. We do not feel that these reports on plasma aldosterone levels alter our findings and conclusions that the adrenal gland and its steroids modify the excretion of H^+ and NH_4^+ during acidosis for the following reasons: (i) In our repletion studies we used the mineralocorticoid DOCA instead of aldosterone; (ii) It could be that during metabolic acidosis there is a generalized release of steroids leading to an increase in total circulating steroids which act on the urinary bladder; and (iii) It is possible that the acidosis results in a change in sensitivity of the receptor in toad bladder for mineralocorticoids. These last two items are only interesting speculation at the present time. It is worthwile to note in support of reason (ii) above, the Quintanilla *et al.* (12) have shown an increase in both aldosterone and cortisol in dogs during metabolic acidosis.

Our results shown in Table II demonstrate that the adrenal gland plays an important role in the excretion of H^+ and NH_4^+ in the acidotic toad. These findings confirm reports by Welbourne (13) and Hulter *et al.* (14) that Adx animals show depressed urinary ammonium and acid excretion. The significant decrease seen in both H^+ and NH_4^+ excretion indicates that one of the secretory products of the adrenal may be responsible for the stimulation of H^+ and NH_4^+ excretion seen during acid base changes in the acidotic animal.

Glucocorticoid-repleted (CT, Dexa) Adx toads (Table III) did not return the excretion rate of NH_4^+ to control levels whereas mineralcorticoid-repleted (DOCA) Adx toads did return to the control excretion rate. This increase in excretion rate in DOCA-replete animals represents a 260% rise above Adx, nonreplete rate. The fact that in the present experiments the NH_4^+ excretory mechanism was sensitive to stimulation only by DOCA may suggest that the response involved has high mineralocorticoid specificity. These results support data presented by Frazier and Zachariah (3) that aldosterone and 17-β estradiol were the only steroid compounds of several tested capable of stimulating NH_4^+ excretion.