Evidence that a Plasma Factor(s) Stimulates H^+ and NH_4^+ Excretion in the Toad Urinary Bladder[1] (36138)

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In vivo and *in vitro* studies from this laboratory (1) have shown that the toad bladder excretes H^+ and NH_4^+, and the H^+ excretion is increased by an NH_4Cl-induced acidosis. This finding raises the question, "What controls the rate of H^+ and NH_4^+ excretion in the toad bladder?"

Rector *et al.* (2, 3) reported that the renal mammalian carbonic anhydrase concentration is a controlling factor in H^+ excretion. They also report pCO_2 to be a controlling factor. Pitts and Lotspeich (4) and Schiess *et al.* (5) reported that the plasma bicarbonate level affects H^+ excretion.

Excretion of NH_4^+ in the mammalian kidney is thought to be controlled by: (i) the urine pH; and (ii) a change in the cellular secretory capacity (6). This change in the secretory capacity is associated with an increase in appropriate renal enzyme concentrations that catalyze ammonia metabolism (7, 8).

The purpose of this study was to determine if an unknown "plasma factor" might alter the H^+ and/or the NH_4^+ excretion in the toad bladder.

Initially, we observed an increase in H^+ and NH_4^+ excretion, produced by plasma from toads in both metabolic and respiratory acidosis, in bladders from nonacidotic toads. To eliminate the factors mentioned above we dialyzed plasma, and extracted plasma with ethyl acetate. By testing these preparations on paired hemibladders, against appropriate control solutions, we avoided changes in the basic cellular enzyme content.

Materials and Methods. The source and the routine care of toads, instrumentation, solutions, the procedure for inducing acidosis and the methods of measuring H^+ and NH_4^+ excretion were as previously described (1). Heparinized plasma was obtained by cardiac puncture from pithed toads. The H^+ flux was calculated using a pK_a for the phosphate buffer pair of 6.50. This value was obtained both empirically in Ringer solution and by calculation (9) to correct for the effect of ionic strength.

Hemibladders from normal nontreated toads were mounted between Lucite chambers. Each chamber was 2 ml in volume. One hemibladder served as a control and the other for the experimental intervention. The mucosal solution was a 0.6 m*M* PO_4 buffered Ringer solution, pH 6.80. The solution contained (m*M*), in addition to the 0.6 m*M* sodium phosphate, NaCl, 114.0; KCl, 3.0; and $CaCl_2$, 0.9. The serosal medium was a 1.5 m*M* PO_4 buffered Ringer solution, pH 7.10, containing 0.61 m*M* glutamine. The indicated additions were made to this solution for experimental procedures. For the lipid extract experiments, ethanol, 1% (v/v), was added to the control and experimental solutions. The pH remained 7.10 after all additions. After a 30 min equilibration period, the 120 min flux determination was begun.

In experiments A and B, a volume of plasma from acidotic toads was dialyzed and the nondialyzable portion was lyophilized, and redissolved in an equivalent volume of Ringer solution. The dialysate was then extracted with ethyl acetate. The ethyl acetate was dried, the residue was dissolved in petroleum ether which was dried, and this residue was redissolved in the appropriate volume of ethanol. This ethanol was then added to a volume of the indicated serosal medium, equal to the original volume of plasma.

In experiments C and D, ethyl acetate extracts were made of acidotic toad plasma,

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and normal toad plasma, respectively. The preparation was the same as above, except that the plasma was extracted directly, rather than being dialyzed.

In the experiments E and F using extracts from dog plasma, heparinized plasma was obtained from a normal mongrel dog, and then from this same dog after administration of 20 g of NH_4Cl over a 48 hr period. The extraction procedure was the same as in experiments C and D above.

Results. Exps. A and B. Table I, Expt. A shows that the lipid extract of the dialyzed fraction of acidotic toad plasma stimulates H^+ excretion ($p<.005$), but did not stimulate NH_4^+ excretion ($p>.20$). The concentration of this extract was 270 μg/ml of Ringer solution. On the other hand in Expt. B we see that the nondialyzable fraction lowered the H^+ excretion ($p>.05$), but increased the NH_4^+ excretion ($p<.05$).

Expts. C and D. Table I, Expt. C shows that the lipid extract of acidotic toad plasma stimulates H^+ excretion ($p<.005$). In 7 out of 10 pairs of hemibladders tested, the H^+ excretion was higher than the corresponding control excretion. In five of the seven bladders stimulated, excretion was more than double the control excretion rate. The NH_4^+ excretion was increased, but not significantly ($p>.05$).

Experiment D shows the effect of a lipid extract from normal toad plasma. As shown, this extract caused no significant increase in either the H^+ or NH_4^+ rates. The concentrations of the extract from the acidotic and normal toad plasma was 80 and 72 μg/ml of Ringer solution, respectively.

Expts. E and F. Expt. E of Table I shows that the lipid extract of acidotic dog plasma stimulated both H^+ and NH_4^+ excretion significantly over the control rates ($p<.005$, $p<.01$, respectively). In these experiments, there was an increased NH_4^+ production, as well as excretion. Expt F shows that a lipid extract of plasma from the same dog before she was made acidotic did not stimulate either H^+ or NH_4^+ excretion ($p>.40$, $p>.05$, respectively). The concentration of the acidotic plasma extract was 236 μg/ml of Ringer solution.

Discussion. This study reports on a newly discovered factor(s) which stimulates H^+ and NH_4^+ excretion in the toad bladder. The factor is obtained by extraction of plasma with lipid solvents. Its activity is assayed *in vitro* in the toad bladder. At similar dosage levels, calculated on the basis of volume equivalents of plasma extracted, the activity was present in plasma from toads in metabolic acidosis, but not in plasma from normal nonacidotic toads. Similarly, it was found in plasma from a dog with metabolic acidosis, but was not present in "normal" plasma obtained from the same dog.

In the lipid-soluble fraction of the dialyzable material, in the direct lipid extract of toad plasma and in the lipid material obtained from the acidotic dog the concentrations used were very low. This was a crude extract of lipid material; only a small fraction of the material is probably active. Therefore, the concentrations used suggest that the substance involved is hormonal in nature. The extract was subject to two drying procedures, thus CO_2 could not be carried with the extract. The solvents used would not dissolve significant quantities of electrolytes in them. These facts support the postulate that a plasma factor, other than ions and CO_2, exerts a control over H^+ and NH_4^+ excretion.

There appears to be a factor present in plasma from metabolic acidotic animals, which also stimulates NH_4^+ excretion. This effect was not due to common chemicals such as NH_4^+, H^+, or CO_2 in solution. The positive effect in nondialyzable fraction of toad plasma, and the positive effect of the lipid extract of dog plasma are the basis for this interpretation. Lipid extracts of plasma from the same dog, before acidosis was induced did not show similar activity.

Evidence suggests that the factor responsible for the NH_4^+ stimulatory effect is separate from that affecting H^+ excretion. The clear separation of the H^+-stimulating effect, and the NH_4^+-stimulating effect was seen in Expts. A and B in the toad. It would seem unlikely that known ammonia precursors such as glutamine, glutaminate, alanine, and glycine would remain after dialysis, although

TABLE I. Effect of Plasma Preparations on H^+ and NH_4^+ Excretion by Toad Bladder.

Excretion (nmoles/100 mg of bladder wet wt × min).

Species	Expt.	Serosal medium	H^+ excretion[a]	NH_4^+ excretion[a]	Mean difference[b] with (p value)[c] H^+ excretion	Mean difference[b] with (p value)[c] NH_4^+ excretion
Toad	A	Ringer solution	8.13	0.70		
		Lipid extract, dialyzable fraction metabolic acidosis plasma[d]	11.25	0.76	3.12 ± 0.86 (<.005)	0.06 ± 0.09 (>.20)
	B	Ringer solution	10.24	0.83		
		Non-dialyzable fraction, metabolic acidosis plasma[d]	7.98	1.00	−2.26 ± 1.07 (>.05)	0.17 ± 0.09 (<.05)
	C	Ringer solution	9.82	0.60		
		Lipid extract metabolic acidosis plasma[d]	15.90	0.71	6.08 ± 1.80 (<.005)	0.11 ± 0.07 (>.05)
	D	Ringer solution	20.59	0.90		
		Lipid extract normal plasma	16.93	0.93	−3.67 ± 1.69 (>.05)	0.30 ± 0.18 (>.40)
Dog	E	Ringer solution	16.34	0.52		
		Lipid extract metabolic acidosis plasma[d]	24.46	0.73	8.12 ± 2.39 (<.005)	0.21 ± 0.07 (<.01)
	F	Ringer solution	34.26	1.47		
		Lipid extract normal plasma[d]	33.05	2.00	−1.21 ± 5.62 (>.40)	0.53 ± 0.33 (>.05)

[a] Each value is an average of 10 experiments.

[b] ± SEM.

[c] Calculated from the mean difference.

[d] In Ringer solution.

this possibility cannot be ruled out altogether. On the other hand, there was a stimulation of NH_4^+ production by the lipid extract of dog plasma. The extraction procedure precludes this being due to such substances as the above-mentioned ammonia precursors.

This study does not disprove the reported observations that H^+ excretion is also effected by pCO_2, HCO_3^- levels and carbonic anhydrase activity. On the contrary we have reported elsewhere (10) that the H^+ excretion in the toad bladder is sensitive to the pCO_2 and that it can be inhibited by acetazolamide. The present study reveals that a lipid factor is also present during acidosis which stimulates H^+ and NH_4^+ excretion in the toad urinary bladder. In addition we have shown that a similar factor is also present in dog plasma during metabolic acidosis which can also stimulate H^+ and NH_4^+ excretion in the urinary bladder.

Summary. H^+ excretion in the toad urinary bladder is increased during metabolic acidosis. A factor can be extracted from plasma of acidotic toads which stimulates H^+ and NH_4^+ excretion by the toad urinary bladder. A factor has also been obtained from plasma of an acidotic dog which stimulates H^+ and NH_4^+ excretion by the toad bladder. The solubility characteristics along with the concentration of the lipid fraction in the media suggest that this factor is hormonal in nature.

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1. Frazier, L. W., and Vanatta, J. C., Biochim. Biophys. Acta **241**, 20 (1971).

2. Rector, F. C., Seldin, D. W., Roberts, A. D., and Smith, J. S., J. Clin. Invest. **39**, 1706 (1960).

3. Rector, F. C., Carter, N. W., and Seldin, D. W., J. Clin. Invest. **44**, 278 (1965).

4. Pitts, R. F., and Lotspeich, W. D., Amer. J. Physiol. **147**, 481 (1946).

5. Schiess, W. A., Ayer, J. L., Lotspeich, W. D., and Pitts, R. F., J. Clin. Invest. **27**, 57 (1948).

6. Pitts, R. F., Fed. Proc., Fed. Amer. Soc. Exp. Biol. **7**, 418 (1948).

7. Pollak, V. E., Mattenheimer, H., DeBruin, H., and Weinman, K. J., J. Clin. Invest. **44**, 169 (1965).

8. Rector, F. C., Seldin, D. W., and Copenhaver, J. H., J. Clin. Invest. **34**, 20 (1955).

9. Knight, A. R., "Introductory Physical Chemistry," p. 159. Prentice-Hall, Englewood Cliffs, NJ (1970).

10. Frazier, L. W., and Vanatta, J. C., Physiologist **14**, 144 (1971)

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