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LUMINAL ACIDIFICATION BY THE PERFUSED RAT CAUDA EPIDIDYMIDIS

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SUMMARY

1. Acid secretion by the rat cauda epididymidis was studied by microperfusion of the epididymal duct and by measuring the pH of the perfusate at a constant pCO_2 using a micro pH sensitive electrode. The rate of acidification was expressed as the rate of fall of intraluminal bicarbonate per cm duct per min.

2. When the cauda epididymal duct was perfused with normal bicarbonate solution, the luminal bicarbonate concentration fell at a rate of $0{\cdot}59 \pm 0{\cdot}39$ n-equiv cm^{-1} min^{-1} (mean $\pm$ S.E., $n = 22$).

3. The rate of luminal acidification was independent of the perfusion rate but was dependent on the concentration of bicarbonate in the perfusion fluid. The rate of fall of luminal bicarbonate increased with increasing bicarbonate concentration and showed saturation at an intraluminal bicarbonate concentration of 25 m-mole/l.

4. Acidification was abolished in the absence of intraluminal sodium ions. This may suggest a linked sodium reabsorption and hydrogen ion secretion.

5. Acidification of the luminal fluid was studied under different acid-base conditions. In animals undergoing metabolic acidosis, the rate of acidification was enhanced, and conversely in animals undergoing metabolic alkalosis, the rate was depressed.

6. Intravenous infusion of acetazolamide into rats at a dose rate of 20 mg/kg.hr markedly inhibited the acidification process. This effect was still observed in animals undergoing metabolic acidosis. Acetazolamide (10^{-5} M) applied luminally was found to have no effect but higher concentration (10^{-4} M) was found to inhibit acidification by 50 %.

7. The role of acidification of the epididymal fluid in sperm maturation was discussed.

INTRODUCTION

We have previously studied the transport functions of the rat cauda epididymidis by microperfusion of the epididymal duct both *in vivo* (Wong & Yeung, 1978; Wong, Au & Ngai, 1978; 1979) and *in vitro* (Wong, Au & Ngai, 1980). We demonstrated that sodium is actively reabsorbed and potassium is secreted into the lumen. Water is reabsorbed isotonically and secondary to sodium transport. These transport processes have many characteristics similar to those of the kidney tubules. In these studies, it was noted that potassium secretion plus chloride reabsorption in the

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epididymal duct could not balance the charge carried by sodium reabsorption. This may infer that hydrogen ions must be secreted (or bicarbonate ions reabsorbed) in order to maintain electroneutrality. These experiments were carried out to see whether acidification takes place in the rat cauda epididymidis perfused *in vivo*, and if so, the basic mechanism of acidification.

METHODS

Male Sprague–Dawley rats weighing between 300 and 400 g were used in all studies. Animals were anaesthetized with I.P. doses of pentobarbitone (50 mg/kg body wet.), tracheostomized, and maintained at 37 °C by direct illumination.

Microperfusion experiments

Details of the procedure for the cannulation and microperfusion of the cauda epididymal duct have been described previously (Wong & Yeung, 1978; Wong, Au & Ngai, 1978, 1979). The only difference was that the epididymides from both sides of the animal were cannulated and perfused simultaneously. The perfused segment of the epididymis was about 18 cm long. Perfusion was carried out at two rates. They were 1·3 μl./min (slow) and 2·3 μl./min (fast) respectively. In most experiments, the slow rate was adopted. After a pre-equilibration period of 40 min, the perfusate was collected over 40 min. When perfused at the slow rate, the perfusate had a volume of about 40 μl. When the perfusion solution was changed, samples were collected only after sufficient time had elapsed for washout of catheter dead space.

Different perfusion fluids were used with electrolyte content set out in Table 1. In most experiments, the ducts were perfused with normal bicarbonate solution (solution A). All solutions contained a trace quantity of [^{3}H]inulin to mark net water flux (Wong & Yeung, 1978).

TABLE 1. Composition of solutions (m-mole/l.)

	Normal HCO_3^- solution	Na$^+$-free	Different HCO_3^- solution**				
	A	B	C	D	E	F	G
NaCl	118	—	143	136	127	118	106·6
KCl	4·7	4·7	4·7	4·7	4·7	4·7	4·7
Choline Cl	—	118	—	—	—	—	—
$CaCl_2$	2·56	2·56	2·56	2·56	2·56	2·56	2·56
$MgSO_4$	1·13	1·13	1·13	1·13	1·13	1·13	1·13
$NaHCO_3$	25	—	—	7	16	25	36·4
Choline HCO_3	—	25	—	—	—	—	—
pH	7·4	7·4	—	—	—	—	—
Gas*	O_2/CO_2	O_2/CO_2	—	—	—	—	—
Calculated osmolarity (m-Osmole)	307	307	305	305	305	305	305

* O_2/CO_2 refers to 95% O_2 and 5% CO_2.

** These solutions were equilibrated with air and had pH ranging from 7 to 8.

Treatment

In a group of experiments, the acid-base status of the animals were altered. Acute metabolic alkalosis was induced by intravenous infusion of 5% sodium bicarbonate solution at a rate of 0·1 ml./min. At least 1 hr elapsed between the start of the infusion and microperfusion of the epididymal duct. A state of metabolic acidosis was achieved by keeping them for at least 3 days on a control diet to which 4% calcium chloride was added, and their water supply was sub-

stituted by a solution containing 70 mM calcium chloride. In addition, during the experiment, they received a solution of ammonium chloride (150 m-equil/l.) at a rate of 0·1 ml./min through the femoral vein (Giebisch, Malnic, de Mello & Mello Aires, 1977).

Administration of acetazolamide

In another group of experiments, the effect of a carbonic anhydrase inhibitor, acetazolamide, was studied. Acetazolamide was administered to rats under two experimental conditions. In one group, rats were given a priming dose of 20 mg/kg by I.V. injection, and then a maintenance dose of 20 mg/kg.hr in isotonic sodium bicarbonate solution through the femoral vein. Infusion was at a rate of 0·1 ml./min. This method was found by Giebisch *et al.* (1977) to maintain the plasma bicarbonate at near normal levels. In another group of rats, the effect of acetazolamide was studied under an acidotic condition. The animals, after given a priming dose of 20 mg/kg, were infused with acetazolamide in ammonium chloride solution (150 m-equiv/l.) at a dose rate of 20 mg/kg.hr (infusion rate 0·1 ml./min). This treatment resulted in a metabolic acidosis.

Blood pH was measured anaerobically in samples collected from the femoral artery midway through the perfusion using a Radiometer micro pH electrode (PHM 64, BSM 2, MK 2). The plasma was then equilibrated with two different gas mixtures (4% carbon dioxide in oxygen and 8% carbon dioxide in oxygen) and the pH was measured under these conditions. The plasma pCO_2 and bicarbonate concentration were read from the Siggaard-Andersen normogram.

Analysis

The radioactivity of [^{3}H] inulin was counted in a scintillation counter (Beckman LS-250) and the net water flux was calculated from the inulin ratio (i.e. [^{3}H]inulin in perfusate over [^{3}H]inulin in the initial perfusion solution) as previously described (Wong & Yeung, 1978). Sodium and potassium concentrations were measured by flame photometry (Zeiss, PF 5) and chloride by chloridometer (Buchler-Cotlove). The pH of the perfusates (40 μl.) were measured using a micro pH probe (Radiometer PHM64, BMS 2, Mk 2) after equilibration with 5% carbon dioxide at 37 °C. Bicarbonate concentrations in the perfusates were determined from the Henderson-Hasselbach equation:

$$pH = 6{\cdot}1 + \log \frac{HCO_3^-}{0{\cdot}03\, pCO_2}$$

Acidification of the perfusate was expressed as the rate of bicarbonate loss by 1 cm duct per min. Transductal fluxes of sodium, potassium chloride were calculated from the knowledge of the perfusion rate after correction for net water fluxes and the initial and final electrolyte concentrations in the perfusion solutions. The net fluxes were expressed in n-equiv cm^{-1} min^{-1} as previously described (Wong & Yeung, 1978).

RESULTS

Basal rate of luminal acidification

When the rat cauda epididymidis was perfused with normal bicarbonate solution (solution A, Table 1). The pH of the perfusates fell. The bicarbonate concentration calculated by the Henderson-Hasselbach equation was found to be significantly less than the initial solution (25 mM). At the perfusion rate of 1·3 μl./min, the perfusate had a bicarbonate concentration of 19·42 ± 0·85 m-mole/l. (mean ± S.E., $n = 8$). After correction of the net water flux, bicarbonate was found to fall at a rate of 0·52 ± 0·053 n-equiv cm^{-1} min^{-1} (mean ± S.E., $n = 8$). At faster rate of perfusion (2·4 μl./min), the perfusate had a bicarbonate concentration of 22·61 ± 0·54 m-mole/l. (mean ± S.E., $n = 6$). Calculation showed that rate of fall of luminal bicarbonate was at a rate of 0·65 ± 0·077 n-equiv cm^{-1} min^{-1} (mean ± S.E., $n = 6$). This values were insignificantly different from those obtained at the slow rate of perfusion. This

experiment showed that luminal acidification as measured by a fall in the luminal bicarbonate was independent of the perfusion rate (Table 2).

Effect of different bicarbonate concentrations

To see whether the acidification in the rat cauda epididymidis varies with the bicarbonate concentration in the lumen, the ducts were perfused with solutions containing various bicarbonate concentrations (solutions C to G, Table 1). Acidification of the lumen (fall in luminal bicarbonate) increased with increasing bicarbonate concentration and reached saturation at an intraluminal bicarbonate concentration of 25 m-mole/l. (Fig. 1). At zero bicarbonate concentration, there was a net gain of bicarbonate in the ductal lumen. The rates of net sodium and water transport across the epididymal duct were found to be independent of the bicarbonate concentration (Fig. 1). At zero bicarbonate concentration, the reabsorption of sodium and water was slightly depressed (by 15 %). However, this change was not significant statistically.

TABLE 2. Effect of perfusion rate on the rates of net electrolyte and water transport and acidification by the perfused rat cauda epididymidis

Rate of perfusion (μl.min^{-1})	H_2O reabsorbed (nl.cm^{-1}min^{-1})	Na^+ reabsorbed	Cl^- reabsorbed (n-equiv cm^{-1} min^{-1})	Acidification	K^+ secreted
1·3	13·8 ± 1·33 (n = 8)	1·91 ± 0·18 (n = 8)	1·06 ± 0·13 (n = 8)	0·52 ± 0·05 (n = 8)	0·13 ± 0·02 (n = 8)
2·4	13·1 ± 2·0 (n = 6)	1·78 ± 0·29 (n = 6)	1·23 ± 0·24 (n = 6)	0·65 ± 0·08 (n = 6)	0·12 ± 0·02 (n = 6)

Acidification is expressed as the rate of fall of luminal bicarbonate concentration.
Each value shows the mean ± S.E. The number of experiments is shown in parentheses.

Reabsorption of chloride ions was found to be enhanced when acidification was abolished at zero bicarbonate concentration. The rate of chloride reabsorption at zero bicarbonate was significantly higher than that observed at 25 m-mole/l. bicarbonate ($P < 0·025$) (Fig. 1). This suggests that a reciprocal relationship might exist between the chloride reabsorption and luminal acidification by the perfused rat cauda epididymidis.

Effect of removal of intraluminal sodium ions

When sodium ions were completely removed and replaced by choline in the perfusion fluid (solution B, Table 1), the reabsorption of sodium and water and secretion of potassium were abolished (Table 3). This confirmed previous results (Wong & Yeung, 1978). Under this condition, there was no acidification of the luminal fluid (Table 3). This indicates that acidification was dependent on intraluminal sodium ions.

Effect of acid-base disorder

Table 4 shows the blood pH and plasma bicarbonate level in different groups of animals. These values were significantly increased in metabolic alkalosis and decreased in metabolic acidosis.

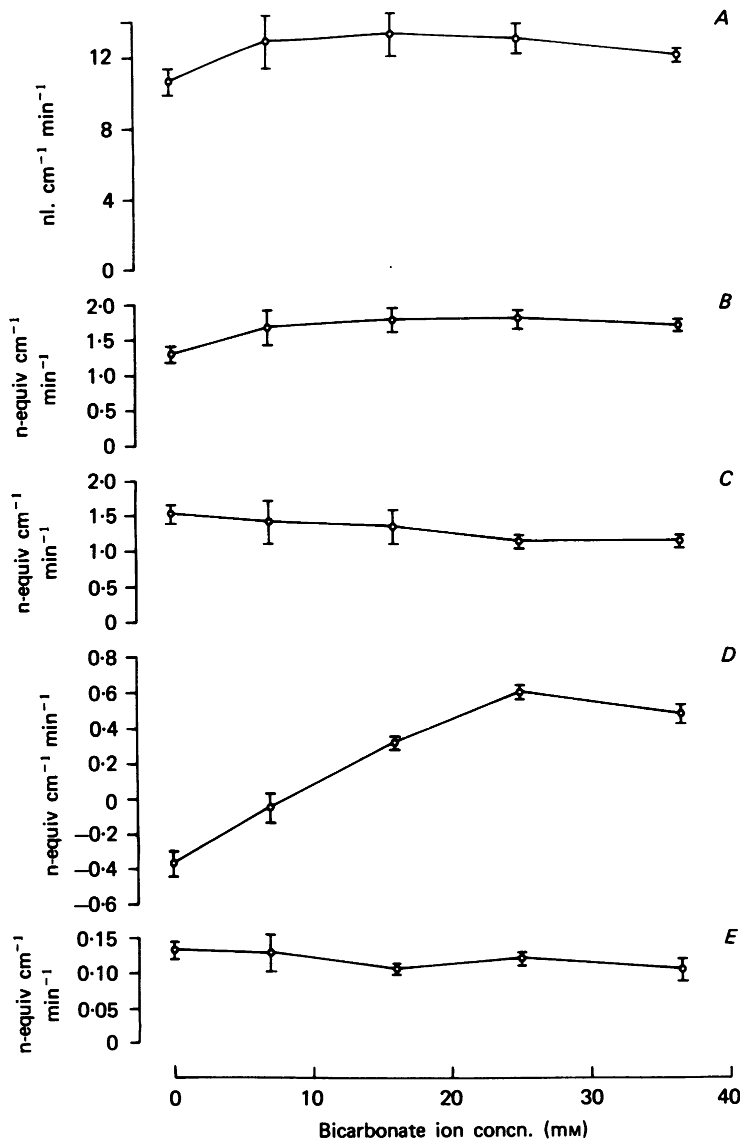

Fig. 1. Dependence on the intraluminal bicarbonate concentration of the rate of (*A*) net water reabsorption, (*B*) net sodium reabsorption, (*C*) net chloride reabsorption, (*D*) acidification, and (*E*) net potassium secretion by the rat cauda epididymidis. Acidification is expressed as the rate of fall of luminal bicarbonate. A negative value in the acidification indicates a net gain of bicarbonate in the ductal lumen. Each point shows the mean ± S.E. of eight to twenty-two determinations. The water, sodium, chloride, bicarbonate and potassium values were obtained from the same samples from eight to twenty-two experiments.

Under condition of acute metabolic acidosis, the rate of acidification by the perfused rat cauda epididymidis was elevated ($P < 0.05$). This was accompanied by an insignificant decrease (about 20 %) of net chloride reabsorption. The reabsorption of sodium and water and secretion of potassium were however unaffected (Table 4).

During acute metabolic alkalosis, luminal acidification was completely suppressed ($P < 0.001$). The net reabsorption of chloride showed a significant increase over the control ($P < 0.025$). The net fluxes of sodium, water and potassium were also without effect (Table 4).

TABLE 3. Effect of sodium ion removal on the rates of net electrolyte and water transport and acidification by the perfused rat cauda epididymidis

Treatment	H_2O reabsorbed ($nl.cm^{-1}min^{-1}$)	Na^+ reabsorbed	Cl^- reabsorbed	Acidification	K^+ secreted
			(n-equiv cm^{-1} min^{-1})		
Control	12.8 ± 0.85 ($n = 22$)	1.74 ± 0.13 ($n = 22$)	1.10 ± 0.09 ($n = 22$)	0.59 ± 0.04 ($n = 22$)	0.12 ± 0.01 ($n = 22$)
Na^+-free solution	0.94 ± 1.10* ($n = 7$)	-0.37 ± 0.03* ($n = 7$)	0.32 ± 0.17** ($n = 7$)	-0.23 ± 0.03* ($n = 7$)	0.08 ± 0.01** ($n = 7$)

Acidification is expressed as the rate of fall of luminal bicarbonate.

Each value shows the mean ± S.E. with the number of experiments shown in parentheses. Statistical significance was tested against the control values using Student's t test. *$P < 0.001$; **$P < 0.025$. A negative value in sodium reabsorption indicates a net secretion into the ductal lumen. A negative value in acidification indicates a net gain of luminal bicarbonate.

Effect of acetazolamide

When acetazolamide was infused into the animals in isotonic sodium bicarbonate (see Methods), the plasma bicarbonate concentration and the pH were near normal levels and luminal acidification was depressed by about 80 % (Table 4). The action of the inhibitor was also studied under an acidotic condition (see Methods). The acidification rate under this condition was inhibited by about 55 % ($P < 0.001$). It is concluded that acidification of the lumen of the rat cauda epididymidis is dependent on the carbonic anhydrase activity. Although luminal acidification was markedly reduced by acetazolamide, the transport of sodium, water and potassium across the cauda epididymal duct was not affected.

In another experiment, acetazolamide was added directly to the luminal perfusion fluid. At 10^{-5} M, acetazolamide was found to have no effect on the rate of acidification. However higher concentration (10^{-4} M) resulted in a 50 % inhibition of the acidification rate (Table 4).

DISCUSSION

These experiments show that the rat cauda epididymidis apart from reabsorbing sodium and water and secreting potassium also acidifies the lumen of the duct. Micropuncture experiments have shown that as the testicular fluid flows down the epididymis, there is a lowering of pH in the fluid (Levine & Marsh, 1971; Levine & Kelly, 1978). Our present results are therefore consistent with these findings.

The basal rate of acidification when expressed as the rate of fall of luminal bicarbonate was 0.586 ± 0.039 n-equiv cm^{-1} min^{-1} (mean ± S.E., $n = 22$). Acidification

TABLE 4. The acid-base parameters and the rates of net electrolyte and water transport and acidification by the perfused rat cauda epididymidis under various experimental conditions

Group	Blood pH	Plasma HCO_3^- (m-equiv/l.)	H_2O reabsorbed (nl. cm^{-1} min^{-1})	Na^+ reabsorbed (n-equiv cm^{-1} min^{-1})	Cl^- reabsorbed (n-equiv cm^{-1} min^{-1})	Acidification (n-equiv cm^{-1} min^{-1})	K^+ secreted (n-equiv cm^{-1} min^{-1})
Control	7·39 ± 0·02 (n = 7)	24·2 ± 1·1 (n = 7)	12·8 ± 0·85 (n = 22)	1·74 ± 0·13 (n = 22)	1·10 ± 0·09 (n = 22)	0·59 ± 0·04 (n = 22)	0·12 ± 0·01 (n = 22)
Acute metabolic acidosis	7·25 ± 0·03** (n = 5)	20·0 ± 1·5**** (n = 5)	13·1 ± 1·29 (n = 10)	1·71 ± 0·21 (n = 10)	0·82 ± 0·12 (n = 10)	0·75 ± 0·08**** (n = 10)	0·14 ± 0·02 (n = 10)
Acute metabolic alkalosis	7·57 ± 0·03** (n = 7)	40·4 ± 4·7** (n = 7)	13·6 ± 1·16 (n = 13)	1·78 ± 0·16 (n = 13)	1·50 ± 0·16*** (n = 13)	0·09 ± 0·08* (n = 13)	0·13 ± 0·01 (n = 13)
Acetazolamide (i.v. infusion in $NaHCO_3$)	7·32 ± 0·02 (n = 9)	23·2 ± 2·10 (n = 9)	12·7 ± 0·70 (n = 11)	1·75 ± 0·14 (n = 11)	1·57 ± 0·11** (n = 11)	0·13 ± 0·06* (n = 11)	0·11 ± 0·01 (n = 11)
Acetazolamide (i.v. infusion in NH_4Cl)	7·04 ± 0·05* (n = 3)	15·1 ± 1·62** (n = 3)	12·1 ± 0·48 (n = 5)	1·64 ± 0·09 (n = 5)	1·41 ± 0·13 (n = 5)	0·26 ± 0·07* (n = 5)	0·13 ± 0·02 (n = 5)
Acetazolamide 10^{-5} M (luminal application)	—	—	13·9 ± 1·00 (n = 7)	1·96 ± 0·16 (n = 7)	1·41 ± 0·12 (n = 7)	0·53 ± 0·04 (n = 7)	0·10 ± 0·01 (n = 7)
Acetazolamide 10^{-4} M (luminal application)	—	—	13·5 ± 0·60 (n = 7)	1·82 ± 0·12 (n = 7)	1·28 ± 0·10 (n = 7)	0·30 ± 0·04* (n = 7)	0·11 ± 0·01 (n = 7)

Acidification is expressed as the rate of fall of luminal bicarbonate.

Each value shows the mean ± S.E. The number of observations is shown in parentheses. Statistical significance was tested against the control values by student's t test. * $P < 0·001$; ** $P < 0·005$; *** $P < 0·025$; and **** $P < 0·05$.

may result from hydrogen ion secretion *per se* or bicarbonate or hydroxide ion reabsorption. Our method of measurement does not permit a distinction to be made between these processes.

In the rat cauda epididymidis, acidification was independent of the perfusion rate but was dependent on the bicarbonate concentration in the lumen. During perfusion with a bicarbonate solution above 7 m-mole/l., there was a net acidification of the lumen. The rate of acidification exhibited saturation at an intraluminal bicarbonate concentration of 25 m-mole/l. (Fig. 1).

Acidification by the rat cauda epididymidis was found to be affected by the acid-base status of the animals. During a metabolic acidosis induced by infusion of ammonium chloride, the acidification rate was enhanced. Conversely, infusion of sodium bicarbonate to produce a metabolic alkalosis resulted in a fall in the acidification rate. These responses to acid-base disorder are very similar to those found in the renal tubules (Pitts, 1964, 1974).

The present study also shows that acetazolamide, the carbonic anhydrase inhibitor, when given intravenously markedly reduced the rate of acidification of the luminal fluid (Table 4). This effect is independent of the metabolic state of the animals. Since carbonic anhydrase catalyses the hydration of carbon dioxide to carbonic acid. This reaction seems to be the rate limiting step in the acidification process. Using histochemical and biochemical techniques, Cohen, Hoffer & Rosen (1976) have demonstrated the presence of carbonic anhydrase in the rat epididymis. This enzyme is required for optimal hydrogen or bicarbonate ion transport in most tissues (Maren, 1967).

It is proposed that in the kidney tubules, acidification of the filtrate is achieved by secretion of hydrogen ions across the luminal membrane in exchange with sodium ions (Pitts & Alexander, 1945; Pitts, 1964; Rector, 1971). Similar mechanism may operate in the rat cauda epididymis. We found that when sodium ions were removed from the lumen, acidification of the luminal fluid was abolished. This observation is consistent with a coupled sodium ion–hydrogen ion exchange pump in the luminal membrane. When sodium ions were removed from the perfusion solution, no linked sodium-for-hydrogen ion exchange was possible, providing a plausible explanation for inhibition of hydrogen ion secretion in the absence of intraluminal sodium.

In conclusion, our present experiments demonstrate that acidification takes place in the perfused rat cauda epididymidis. This may result from hydrogen ion secretion or bicarbonate reabsorption by the duct. Acidification depends on sodium reabsorption, inhibited by acetazolamide and affected by the acid-base status of the animals. In these respects, it has many characteristics similar to that occurring in the kidney tubules. Since hydrogen ions and bicarbonate ions have been shown to affect sperm motility and metabolism (Emmens, 1947; Blackshaw & Emmens, 1951; Mounib & Eisan, 1968; Dacheux, O'Shea & Paquignon, 1979), acidification of the epididymal fluid may have an important role in the maturation and motility of spermatozoa *in vivo*.

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