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Effects and Interactions of Furosemide and Acetazolamide on Tubular Function in Rat Kidney

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Furosemide and acetazolamide effects on tubular function in rat kidney have been studied by micropuncture. Furosemide produced a marked rise in fractional proximal fluid reabsorption when urine loss was not replaced, and sodium excretion rose significantly indicating a distal effect. If urinary losses were replaced proximal fractional reabsorption was depressed and fractional sodium excretion increased more than 60 %.

After replacing urinary losses, acetazolamide had a greater depressive effect on proximal tubular fluid reabsorption than furosemide but sodium excretion values were about 1/3 of those obtained with furosemide. Superimposition of one drug during the action of the other resulted in potentiation of proximal inhibition, suggesting a different mechanism of action.

The changes observed in potassium excretion are of great interest. Separately, furosemide or acetazolamide produced kaliuresis. When furosemide was administered during acetazolamide diuresis, however, potassium excretion was reduced despite the sharp rise in sodium excretion.

It is well known that acetazolamide exerts its diuretic effect through inhibition of carbonic anhydrase which leads to a reduction of sodium hydrogen exchange principally across the luminal border of proximal tubular cells (13). By contrast furosemide, although a sulfonamide, appears to have carbonic anhydrase inhibitory effect only *in vitro* (1).

On the other hand, furosemide may inhibit renal Na^+, K^+, ATPase (12-17) while no such effect has been demonstrable for acetazolamide (14). Furthermore, furosemide appears to have important effects on cellular metabolism (11-19) which may contribute to inhibition of tubular transport.

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The principal site in the nephron where these two agents act is also different. Acetazolamide has a major effect in the proximal tubule (10-15) while it exerts no direct action in Henle's loop (16). Furosemide, on the other hand, exerts its major inhibitory effect on reabsorption in the ascending limb of Henle's loop (6-18). An action in the proximal tubule has also been observed in both clearance and micropuncture studies, but prevention of extracellular fluid volume depletion by exact replacement of urinary sodium and water losses is necessary to insure demonstration of an effect at this site (2-7). A reduction in sodium reabsorption has also been adduced to occur in the collecting duct when large doses of the diuretic are administered (8).

It has been tried to compare the renal effect of furosemide and acetazolamide hoping to elucidate further the mechanism of action of both agents and also to determine the magnitude of the furosemide proximal effect in the presence of almost complete inhibition of carbonic anhydrase.

Materials and Methods

The experiments were carried out in male Wistar rats weighing between 240 and 350 g. Animals were fasted for 12-14 h before the experiment and allowed water *ad libitum*. They were anesthesized by intraperitoneal injection of sodium pentobarbital (100 mg/kg weight) and a tracheostomy performed. Both external jugular veins were cannulated for the injection of inulin and lissamine green; the femoral artery was catheterized for blood sampling and for registering arterial pressure when necessary; urine was collected from an indwelling bladder catheter. An incision was made below the left costal margin in order to gain access to the kidney and dissect it from the perirenal fat. Once the animal had been prepared in this way it was placed on a lucite table kept at a constant temperature by means of a thermostat; this rested on a large marble table to lessen vibrations. The left kidney was placed in a plastic bowl to keep it from moving and was surrounded by mineral oil at a steady temperature of 37° C. An inulin-saline solution was infused at a speed of 0.015 ml/min by means of a Braun pump so as to achieve plasma concentrations of inulin of about 100 mg/100 ml. An hour later micropuncture was carried out. The urinary losses were weighed on a precision balance every 2-4 minutes and replaced with the same quantity of saline solution. Two ml of isotonic saline solution was administered to replace surgical losses. Rectal temperature was monitored by means of a telethermometer.

Tubular fluid samples were obtained from a group of 16 rats during the control period followed by samples after furosemide administration. The diuretic was given in an initial dose of 25 mg/kg weight followed by a maintenance dose of 15 mg/kg weight/hour. In these experiments two protocols were followed: *a*) one in which urinary losses were not replaced; *b*) one in which isotonic saline solution was infused intravenously in quantities equal to urinary losses.

In another group of 10 rats acetozolamide was given at an initial dose of 50 mg/kg weight and at a maintenance dose of 20 mg/kg weight/h with urinary loss replacement.

In a third group of experiments 12 rats received first acetazolamide and then furosemide at the above-mentioned doses; in a fourth group of 10 rats furosemide was first administered followed by acetazolamide. Urinary losses were replaced in both studies.

Inulin in blood, tubular fluid and urine were measured by the method of VUREK and PEGRAM (21). Sodium and potassium concentrations in blood and urine were measured in an IL flame photometer. Arterial pressure was monitored with a

mingograph 34. Statistical analysis was done on an Olivetti 101 calculator. The transit-time was measured by injection of lissamine green, which also permitted the localization of the end of the proximal convolution.

The GFR per nephron was calculated: $(TF/p)_{in} \times$ (tubular flow).

Results

Effect of furosemide on proximal tubular reabsorption (no replacement of urinary losses). Failure to replace urinary losses after furosemide administration resulted in a marked rise in transit time which became almost four times the control value (table I, A). There was a marked increase in TF/P inulin and a reduction in single nephron GFB. This was accompanied by a marked reduction in total GFR, but a significant increase in urine flow and the excretion of sodium and potassium.

Effect of furosemide on proximal tubular reabsorption during careful replacement of urinary losses. Under these circumstances transit time went up as when urine losses were not replaced but the magnitude of the change was less (table I, B). In contrast to the results in table I, TF/P inulin dropped while GFR per nephron and total GFR remained unchanged. Urine volume and U_{Na} V rose but the change was between approximately 80 and 250 %. The change in U_k V was comparable in both cases.

Effect of acetazolamide on proximal tubular reabsorption with replacement of urinary losses. The changes observed with acetazolamide were directionally identical to those with furosemide (table II). However, transit time was prolonged to a lesser extent and (TF/P) inulin reduced to a greater degree than with furosemide. Similarly, the rises in V and U_{Na} V were less with acetazolamide than with furosemide. While the absolute value for U_k V was greater for acetazolamide, the per cent change induced by furosemide was greater.

Effect of acetazolamide and acetazolamide plus furosemide with replacement of urinary losses. It should be noted that all values during the acetazolamide diuresis are comparable to those shown in table II. This illustrates that replacement of urinary losses was adequate. Superimposition of furosemide on an acetazolamide diuresis led to further reduction in (TF/P) inulin and an inhibition

Table I. *Effect of furosemide on proximal tubular reabsorption and total excretion.* Mean ± S.D.; N.S., no significant; n = number of rats.

Period	Transit time Seconds	TF/P_{in}	GFR nephron nl/min/kg	Urine volume µl/min/kg	Total GFR ml/min/kg	U_{Na} V µEq/min/kg	U_K V µEq/min/kg
			A) *Without replacement of urinary losses* (n = 6)				
Control	10.5±0.54	1.96±0.31	124±15.3	14.8± 4.6	3.35±1.15	5.4± 1.4	18.5± 5.9
Exper.	38.2±1.78	2.82±0.32	59±16.6	97 ±20.3	1.60±0.64	191.9±48.4	51.1±11.8
p	< 0.001	< 0.0025	< 0.001	< 0.0025	< 0.001	< 0.001	< 0.001
			B) *With replacement of urinary losses* (n = 6)				
Control	11.3±1.2	1.97±0.24	110.6±17.1	20.5± 7.6	3.35±1.18	5.25±1.97	15.6±2.8
Exper.	25.3±3.7	1.73±0.16	124.3±16.7	311.8±76.5	3.05±0.88	375.6 ±65.9	60.3±8.7
p	< 0.001	< 0.025	N.S.	< 0.001	N.S.	< 0.001	< 0.001

Table II. *Effect of acetazolamide on proximal tubular reabsorption and total excretion with replacement of urinary losses.*
Mean ± S.D.; N.S. no significant; number of rats, 10.

Period	Transit time Seconds	TF/P_{in}	GFR nephron nl/min/kg	Urine volume µl/min/kg	Total GFR ml/min/kg	U_{Na} V µEq/min/kg	U_K V µEq/min/kg
Control	12.1±0.7	2.07±0.28	114.1±24.7	26.1± 9.6	3.3 ±1.3	4.8± 1.5	29.6±11.5
Exper.	18.6±1.5	1.59±0.17	109.6±20.3	110.1±22.8	3.01±1.6	120.3±17.8	94.3±16.8
p	< 0.001	< 0.0025	N.S.	< 0.001	N.S.	< 0.001	< 0.001

Table III. *Combined effect of acetazolamide and furosemide on proximal tubular reabsorption.*
Mean ± S.D.; N.S., no significant; n = number of rats.

	Transit time Seconds	TF/P_{in}	GFR nephron nl/min/kg	Urine volume µl/min/kg	Total GFR ml/min/kg	U_{Na} V µEq/min/kg	U_K V µEq/min/kg
			A) *Acetazolamide and furosemide* (n = 12)				
Acetazolamide	17.5±1.6	1.63±0.23	99.5±16.7	108.6±19.7	3.6±1.0	119.0±21.0	96.6±15.7
Acetazolamide + furosemide	29.3±1.9	1.37±0.11	115.5±14.0	290.6±69.8	3.3±1.2	358.0±48.8	79.5± 8.8
p	< 0.001	< 0.025	N.S.	< 0.001	N.S.	< 0.001	< 0.025
			B) *Furosemide and acetazolamide* (n = 10)				
Furosemide	29.6±1.86	1.64±0.1	123.5±15.1	335.3±59.5	3.15±1.04	347.6±87.8	61.0±21.8
Furosemide + Acetazolamide	30.6±2.7	1.30±0.09	103.1±19.1	341.6±86.7	3.08±0.99	423.5±135.4	91.3±20.1
p	N.S.	< 0.001	< 0.025	N.S.	N.S.	N.S.	< 0.025

of kaliuresis although sodium excretion rose significantly (table III, A).

Effect of furosemide and of furosemide plus acetazolamide with replacement of urinary losses. Superimposition of acetazolamide on a furosemide diuresis led to further reduction in (TF/P) inulin and in GFR per nephron. In addition, U_k V rose significantly. No appreciable change could be detected in transit time, urine flow, total GFR or U_{Na} V (table III, B).

Blood hematocrit and plasma, sodium, potassium, chloride and bicarbonate did not change significantly between control and experimental periods in any of the groups.

Discussion

The present results demonstrate that acute volume depletion caused by furosemide masks its effect on proximal tubular reabsorption (3, 4, 7). In fact, proximal

fractional reabsorption after furosemide administration was enhanced, as indicated by a marked rise in TF/P inulin ratio, when urine loss was not replaced. A principal cause for this finding is the marked reduction in total and single nephron GFR. Furthermore, the rise in plasma proteins which must have been caused by acute volume contraction could have played a role in enhancing reabsorption (5). Despite this absolute and fractional sodium, excretion rose significantly indicating a predominant effect of the diuretic on distal tubular reabsorption, at least in superficial nephron. Nevertheless, it is of great interest that fractional sodium excretion in this group of animals was comparable to that observed in the group receiving urinary loss replacement in which TF/P inulin dropped. The magnitude of the fractional excretion (60 %) strongly suggests that inhibition of proximal tubular reabsorption in deeper nephrons took place. Levels of sodium excretion such as those observed here have been utilized in clearance studies to infer a proximal site of action for furosemide (18, 20). From the present studies it is clear that the conclusion is correct.

Although the acetazolamide effects were not examinated without replacing urinary losses, from the magnitude of absolute sodium excretion, it may be predicted that acute volume depletion could have blunted the diuresis significantly.

Acetazolamide does not appear to have a direct effect in the loop of Henle (16) so that, despite greater depressive effects on proximal tubular reabsorption than furosemide, the magnitude of the diuresis was about the same as when urinary replacement was not carried out with furosemide and 1/3 of values obtained with furosemide plus replacement. This difference in the site of action of the two diuretics is more dramatically borne out by the experiments where one drug was superimposed on the other. When furosemide was administered during acetazolamide diuresis the degree of proximal inhibition was similar to that of the reverse experiment, yet a greater diuresis resulted during the former than in the latter experiments. In addition to the fact that acetazolamide has no loop effect, its reduction of GFR/nephron must have been a factor.

The combined action of the diuretics also has a bearing on the mechanism of action of the two drugs. If furosemide has a carbonic anhydrase inhibitory effect, it must be of very small magnitude since administration of acetazolamide still resulted in depression of proximal tubular reabsorption. On the other hand, furosemide superimposition on acetazolamide diuresis also depressed proximal reabsorption further. This effect was of the same magnitude as when acetazolamide was superimposed on furosemide yet, by itself, furosemide's effect was less than acetazolamide. It could be suggested that furosemide, even if it has some action on carbonic anhydrase, must interfere with another determinant of reabsorption such as luminal cell membrane permeability or cellular metabolism. An effect on Na^+, K^+, ATPase in the proximal tubule has not been shown for either furosemide or acetazolamide (4).

The changes in K^+ excretion in these experiments are of great interest. Under all circumstances in which each diuretic was administered by itself, kaliuresis resulted. In addition, acetazolamide superimposed on furosemide also led to an increase in K^+ excretion. When furosemide was administered during acetazolamide diuresis, however, it resulted in a reduction in $U_k V$. The kaliuresis induced by furosemide is the result of the increased distal tubular delivery and the elevation of luminal sodium concentration which takes place. Acetazolamide increases K^+ excretion by altering the acid base status of the cell. Acetazolamide enhances peritubular cell membrane uptake of potassium (22) allowing passive secretion into

the lumen. Furosemide, therefore, may have reduced peritubular cell membrane uptake and passive K^+ secretion by altering cell permeability. Another possibility is that the sudden increment in urine flow produced by furosemide led to a fall in distal bicarbonate concentration, lowering luminal fluid pH and reducing K^+ secretion. These possibilities deserve further study.

Resumen

Se han estudiado los efectos de la furosemida y la acetozalamida sobre la función tubular en el riñón de rata con técnicas de micropunción. La furosemida produjo una marcada elevación en la resorción fraccional del fluido proximal cuando las pérdidas urinarias no fueron reemplazadas y la excreción de sodio se elevó significativamente indicando un efecto distal. Cuando se repusieron las pérdidas urinarias la resorción fraccional proximal disminuyó y la excreción fraccional de sodio aumentó más del 60 %.

Con reposición de pérdidas urinarias, la acetazolamida tuvo un efecto depresivo mayor que la furosemida sobre la resorción del fluido proximal, si bien la excreción de sodio fue alrededor 1/3 de los valores obtenidos con la furosemida. La administración de una droga cuando la otra estaba actuando dio lugar a un aumento de la inhibición proximal sugiriendo un mecanismo de acción diferente.

La furosemida y la acetazolamida aisladas produjeron aumento de la excreción de potasio. Sin embargo, cuando la furosemida se administró durante la acción de la acetozolamida la excreción de potasio disminuyó a pesar de la gran elevación en la excreción de sodio.

References

1. Baer, J. E. and Beyer, K. H.: *Ann. Rev. Pharmacol.*, **6**, 261-292, 1966.
2. Bennett, D. M., Brenner, B. M. and Berliner, R. W.: *J. Clin. Invest.*, **47**, 203-216, 1968.
3. Berliner, R. W., Dirks, J. H. and Cirksena, W. J.: *Ann. N.Y. Ac. Sci.*, **139**, 424-432, 1966.
4. Brenner, B. M., Keimowitz, R. I., Wright, F. S. and Berliner, R. W.: *J. Clin. Invest.*, **48**, 290-300, 1969.
5. Brenner, B. M., Falchuk, K. H., Keimowitz, R. I. and Berliner, R. W.: *J. Clin. Invest.*, **48**, 1519-1531, 1969.
6. Burg, M., Stoner, L., Cardinal, J. and Green, N.: *Amer. J. Physiol.*, **225**, 119-124, 1973.
7. Burke, T. J., Robinson, R. R. and Clapp, J. R.: *Kidney Internat.*, **1**, 12-18, 1972.
8. Dirks, J. H. and Seely, J. F.: *Amer. J. Physiol.*, **219**, 114-121, 1970.
9. Knox, F. G., Wright, F. A., Howards, S. S. and Berliner, R. W.: *Amer. J. Physiol.*, **217**, 192-198, 1969.
10. Kunau, R. T.: *J. Clin. Invest.*, **51**, 294-306, 1972.
11. Landon, E. J. and Forte, L. R.: *Ann. Rev. Pharmacol.*, **11**, 171-188, 1971.
12. Murphy, J. C. and Bader, H.: *Pharmacol.*, **10**, 162-169, 1968.
13. Pitts, R. F. and Alexander, R. S.: *Amer. J. Physiol.*, **144**, 239-245, 1945.
14. Radke, H. W. Rumrich, G., Kinne-Saffran, R. and Ullrich, K.: *Kidney Internat.*, **1**, 100-105, 1972.
15. Rector, F. C., Jr., Carter, N. W. and Seldin, D. W.: *J. Clin. Invest.*, **44**, 278-290, 1965.
16. Rosin, J. M., Ktaz, M. A., Rector, F. C., Jr. and Seldin, D. W.: *Amer. J. Physiol.*, **219**, 1731-1738, 1970.
17. Schmidt, U. and Dubach, U. C.: *Nephron.*, **7**, 447-458, 1970.
18. Seldin, D. W., Eknoyan, G., Suki, W. N. and Rector, F. C., Jr.: *Ann. N. Y. Acad. Sci.*, **129**, 328-343, 1966.
19. Suki, W. N., Eknoyan, G. and Martínez-Maldonado, M.: *Ann. Rev. Pharmacol.*, **13**, 91-106, 1973.
20. Suki, W. N., Rector, F. C., Jr. and Seldin, D. W.: *J. Clin. Invest.*, **44**, 1458-1469, 1965.
21. Vurek, G. and Pegram, S. E.: *Analyt. Biochem.*, **16**, 409-419, 1966.
22. Wiederholt, M., Sullivan, W. J. and Giebisch, G.: *J. Gen. Physiol.*, **57**, 495-499, 1971.