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EFFECT OF URINARY pH AND URINE FLOW RATE ON PROSTAGLANDIN E_2 AND KALLIKREIN EXCRETION BY THE CONSCIOUS DOG

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SUMMARY

1. The effects of urine flow rate and urinary pH on renal prostaglandin E_2 (PGE_2) and kallikrein excretion were investigated in conscious dogs during water deprivation followed by rehydration and under conditions which altered urine pH, but caused similar change in salt and water excretion.

2. When water-restricted dogs were rehydrated in two steps by gavage giving the animals tap water, urine flow increased by 22- and 63-fold with a concomitant increase in PGE_2 excretion by 100 and 318 %, respectively; whereas urinary kallikrein excretion and urine pH did not change significantly.

3. Oral administration of isotonic sodium chloride solution increased urine flow as well as electrolyte excretion without altering urine pH ($6{\cdot}90 \pm 0{\cdot}26$) and PGE_2 excretion.

4. When urine was made alkaline (pH $7{\cdot}79 \pm 0{\cdot}09$) by oral sodium bicarbonate, urine flow and electrolyte excretion were similar to those observed after oral sodium chloride, while renal PGE_2 excretion increased by 66 % ($P < 0{\cdot}05$).

5. When urine was made acidic (pH $5{\cdot}31 \pm 0{\cdot}14$) by oral ammonium chloride, urine flow and electrolyte excretion were similar to the values seen after oral sodium chloride. Urinary PGE_2 excretion, however, was reduced by 46 % ($P < 0{\cdot}05$).

6. After oral fluid loads a positive correlation could be detected between urine pH and urinary PGE_2 excretion ($r = 0{\cdot}854$, $P < 0{\cdot}001$).

7. Urinary kallikrein excretion was not significantly altered by any of the three interventions mentioned above.

8. The present results suggest that, in conscious dogs, urine flow as well as urine pH are important determinants of urinary PGE_2 excretion rates, but not of kallikrein excretion.

* The experiments were conducted in the Department of Physiology, Semmelweis University Medical School, Budapest, Hungary.

INTRODUCTION

Measurement of urinary excretion rate of prostaglandin E_2 (PGE_2) has been widely used as an index of intrarenal prostaglandin production since PGE_2 excreted into the urine is thought to be derived entirely from the kidney (Frölich, Sweetman, Carr, Splawinski, Watson, Anggard & Oates, 1973; Frölich, Wilson, Sweetman, Smigel, Nies, Carr, Watson & Oates, 1975). Recent reports of apparent flow dependence of PGE_2 excretion in the conscious dog (Kirschenbaum & Serros, 1980; Wright, Rosenblatt & Lifschitz, 1981; Fejes-Tóth, Filep & Mann, 1983*a*) and in human (Kaye, Zipser, Hahn, Zia & Horton, 1980; Walker, Brown & Stoff, 1981; Haylor, Lote & Thewles, 1986*a*), however, show that it is necessary to exactly define the conditions under which the excretion of urinary PGE_2 was ascertained in order to use this parameter as an index of renal prostaglandin synthesis. Furthermore, the weak acidic properties of prostaglandins suggest that their excretion rate could be influenced by urinary pH. Although this possibility was mentioned by Frölich, Wilson, Sweetman, Smigel, Nies, Carr, Watson & Oates in 1975, urine pH was rarely, if ever measured in experiments investigating the renal prostaglandin system. Experimental evidence that urine pH *per se* might be an important determinant of urinary PGE_2 excretion in rats (Haylor, Lote & Thewles, 1984) and in man (Haylor *et al.* 1986*a*) has recently been published. As the mechanisms governing the excretion rate of prostaglandins might be different in man, rats and dogs, and as in the dog pH dependency of PGE_2 excretion has not been reported, we thought it might be of interest to examine the relationship between PGE_2 excretion and urine pH in this species. In addition, we determined urinary kallikrein activity since the relationship between urinary PGE_2, kallikrein and urine pH is also poorly understood.

METHODS

Studies were conducted on seven female mongrel dogs weighing between 11 and 18 kg. In order to facilitate bladder catheterization an episiotomy was performed on all animals several weeks before the first experiment. The dogs were trained to stand quietly with the support of loose-fitting slings and were accustomed to the procedures of the experiments. On each animal four different experiments were performed. At least one week was allowed to elapse between different experiments on the same dog. The order of the experiments was randomized. On the morning of the experiments the animals were fitted with an indwelling bladder catheter and with an intravenous cannula. Urine was collected into pre-chilled tubes.

In Experiment 1 animals were studied during spontaneous antidiuresis after 24 h of fasting and water deprivation. After an equilibration period of approximately 2 h, two consecutive urine collections of 30 min each were made. Then the dogs were rehydrated by gavage giving the animals 16 ml/kg body weight of tap water. 40 min later, when urine flow rate had stablized, two consecutive urine collections of 15 min each were made. A further increase in urine flow was achieved by gavage giving the animals two times 35 ml/kg body weight of tap water 30 min apart and subsequent doses equalling the volume of urine excreted. When urine flow had stabilized (120–140 min after the second gavage) two urine collection periods of 15 min each followed. Values obtained in the first–second, third–fourth and fifth–sixth urine collection periods were averaged and were considered as control, experimental 1 and experimental 2 periods, respectively. Experiments 2–4 were begun in normohydrated dogs. Prior to these experiments food was withheld for 16 h but the animals had free access to water. After an equilibration period of approximately 2 h two consecutive urine collections of 15 min each (control) were performed. Immediately after the second period diuresis was produced by gavage giving the animals two times 25 ml/kg body weight

of 0·9% sodium chloride (Experiment 2) or 1% sodium bicarbonate (Experiment 3) or 1% ammonium chloride (Experiment 4) solution 30 min apart. 70 min later two urine collections (15 min in length) were made. Values obtained from the two control and third–fourth periods were averaged.

For PGE_2 determination, an aliquot of urine corresponding to the amount excreted over a period of 10 min for a standardized body weight of 10 kg was removed, acidified to pH 4·0 with ammonium acetate and a tracer amount of ^{3}H-$PGF_{1\alpha}$ was added to monitor procedural losses. The sample was then passed through an Amberlite CG 50 I (Serva, Heidelberg, F.R.G.) column and prostaglandins were eluted with ethanol. The ethanol fraction was dried under N_2 and was redissolved in toluene:ethyl acetate:acetic acid (60:40:0·1 v/v) and applied to a 1 g silicic acid column equilibrated in the same solvent system. The column was washed with 7 ml of the above solvent system and prostaglandins were eluted with 2 × 1·5 ml of toluene: ethyl acetate:ethanol:acetic acid (60:40:30:0·1 v/v). The eluates were dried under N_2, redissolved in ethanol and stored at −20 °C until analysis. Using this procedure the recovery was 84·5 ± 1·6% (n = 102). When urine samples spiked with ^{3}H-$PGF_{1\alpha}$ and ^{14}C-PGE_2 (Amersham) were extracted and chromatographed as described above in no case did the difference between recoveries of ^{3}H-$PGF_{1\alpha}$ and ^{14}C-PGE_2 exceed 4%.

Radioimmunoassay of PGE_2 was performed using specific antiserum (Pasteur Institute, Paris, France) as described in detail previously (Fejes-Tóth *et al.* 1983*a*). This antibody had less than 0·5% cross-reactivity with PGA_2, $PGF_{1\alpha}$, $PGF_{2\alpha}$, 6-keto-$PGE_{1\alpha}$ and PGD_2 Intra- and interassay coefficients of variation were 2·9 and 7·0%, respectively. Values were corrected for individual recovery.

For kallikrein determination an aliquot of urine corresponding to the amount excreted over 2 min and a standardized body weight of 10 kg was removed and dialysed against redistilled water overnight at 0–4 °C. The dialysed urine was lyophylized and redissolved in an appropriate amount of Tris-HCl buffer (pH 8·2, 0·2 M) before assay. Kallikrein activity was measured by an amydolytic assay (Amundsen, Putter, Friberger, Knos, Larsbraten & Claeson, 1979). Intra and interassay coefficients of variation were 3·7 and 7·1%, respectively.

Urine was checked in each experiment for haematuria by Hemaocombistix (Ames). Experiments in which haematuria occurred were excluded from the studies. The pH of each urine sample was measured immediately after the end of urine collection period by a precision pH meter (Radiometer, Copenhagen). Standard methods were used to determine osmolality (freezing point depression) and sodium and potassium concentration (flame photometry).

Statistical evaluation of the data was performed by Friedman's test for two-way analysis of variance by ranks and by the Wilcoxon–Wilcox's test for multiple comparisons (control *versus* experimental 1, control *versus* experimental 2 and experimental 1 *versus* experimental 2 within each parameter of the dehydration– rehydration experiments). The differences in Experiments 2–4 were assessed by Wilcoxon's test for paired observations and by the Mann–Whitney U test for unpaired observations. To investigate correlations Spearman's rank correlation coefficients (r_S) were calculated. A 0·05 level was considered significant for all tests.

RESULTS

As can be seen in Table 1 the two-step rehydration of water-deprived dogs produced marked diuresis with a concomitant increase in urinary PGE_2 excretion. Despite the marked increase in urine flow urine pH as well as kallikrein excretion remained practically unchanged. Further analysis of data obtained during water deprivation and after rehydration revealed that urinary excretion rate of PGE_2 was negatively correlated with plasma osmolality ($r_S = -0{\cdot}618$, $P < 0{\cdot}005$), plasma sodium concentration ($r_S = -0{\cdot}522$, $P < 0{\cdot}01$), and urine osmolality ($r_S = -0{\cdot}809$, $P < 0{\cdot}001$); whereas it was positively correlated with urine flow ($r_S = 0{\cdot}874$, $P < 0{\cdot}001$) and urinary sodium excretion ($r_S = 0{\cdot}439$, $P < 0{\cdot}02$). The urinary excretion rate of kallikrein was not correlated with any of the aforementioned variables.

Means of absolute values of salt and water excretion produced by the three different

TABLE 1. Effects of water deprivation–rehydration and administration of sodium chloride, sodium bicarbonate and ammonium chloride on water and electrolyte excretion, urine pH, prostaglandin E_2 and kallikrein excretion in conscious dogs

	V (ml/min)	U_{osmol} (mosmol/kg H_2O)	Urine pH	$U_{Na}V$ (μM/min)	U_KV (μM/min)	PGE_2 excretion (pg/min)	Kallikrein excretion (mK.U./min)
			Experiment 1, water deprivation–rehydration, $n = 7$				
Control	0·098±0·008	1260±103	6·92±0·26	75±13	23±7	112±20	745±125
Experimental 1	2·180±0·670*	241±91*	6·67±0·17	82±28	20±4	226±41*	756±71
Experimental 2	6·232±0·396†	50±3†	6·70±0·04	96±17	24±8	472±98†	837±115
P	< 0·001	< 0·001	n.s.	n.s.	n.s.	< 0·01	n.s.
			Experiment 2, oral sodium chloride, $n = 6$				
Control	0·146±0·016	907±71	6·90±0·26	58±15	18±3	146±40	720±89
Experimental	4·246±0·758*	336±16*	6·91±0·18	353±35*	109±31*	136±12	810±131
			Experiment 3, oral sodium bicarbonate, $n = 6$				
Control	0·154±0·022	934±134	7·08±0·16	59±17	19±6	144±18	699±51
Experimental	5·086±0·926*	180±14*	7·79±0·09*	347±86*	92±16*	238±40*	753±114
P	n.s.	n.s.	< 0·01	n.s.	n.s.	< 0·05	n.s.
			Experiment 4, oral ammonium chloride, $n = 6$				
Control	0·126±0·020	918±116	7·08±0·24	56±16	18±6	158±26	653±77
Experimental	4·252±0·760*	238±53*	5·31±0·14*	255±30*	105±13*	74±12*	719±64
P	n.s.	n.s.	< 0·005	n.s.	n.s.	< 0·05	n.s.

Values are means ±S.E. of mean. Abbreviations: V, urine flow; U_{osmol}, urinary osmolality; $U_{Na}V$, urinary sodium excretion; U_KV, urinary potassium excretion; K.U., kallikrein unit. V, $U_{Na}V$, U_KV, PGE_2 and kallikrein excretion rate are referred to 1 m^2 of body surface. * $P < 0·05$; † $P < 0·001$; n.s., not significant. P values are the probability levels of the two-way analysis of variance obtained by Friedman's test; P values were obtained by Mann–Whitney's U test for comparing the changes observed in Experiments 3 and 4 with the changes seen in Experiment 2.

oral fluid loads are summarized in Table 1. Control values for all parameters were similar in the three experiments (Tables 1 and 2). The average increase in urine flow produced by the three interventions was similar (29-fold increase in Experiment 2; 33- and 34-fold increase in Experiments 3 and 4, respectively). Urine osmolality

TABLE 2. Effects of water deprivation–rehydration, oral sodium chloride, sodium bicarbonate and ammonium chloride loadings on various plasma variables in the dog

	Plasma		
	Na (mmol/l)	K (mmol/l)	Osmolality (mosmol/kg H_2O)
Experiment 1, water-deprivation–rehydration, $n = 7$			
Control	143±2	3·8±0·1	306±3
Experimental 1	139±2	3·7±0·2	297±3*
Experimental 2	133±3†	3·6±0·6	277±4†
P	< 0·01	n.s.	< 0·001
Experiment 2, oral sodium chloride, $n = 6$			
Control	141±2	3·8±0·1	297±2
Experimental	140±3	3·7±0·2	297±3
Experiment 3, oral sodium bicarbonate, $n = 6$			
Control	141±3	3·8±0·1	299±3
Experimental	141±3	3·7±0·2	299±3
Experiment 4, oral ammonium chloride, $n = 6$			
Control	141±2	3·8±0·2	297±1
Experimental	141±2	3·9±0·2	297±1

Values are means ±S.E. of mean. * $P < 0·05$; † $P < 0·01$ (probability levels obtained by Wilcoxon–Wilcox's test for control *vs.* experimental 1 and control *vs.* experimental 2). *P* values are the probability levels of the two-way analysis of variance obtained by Friedman's test.

decreased significantly in all experiments. The magnitude of the saluretic response was higher after sodium chloride, as sodium and potassium excretion increased on the average by 509 and 505%, respectively. Sodium bicarbonate produced 488 and 384% increases in sodium and potassium excretion, whereas ammonium chloride increased sodium and potassium excretion by 355 and 483%, respectively. Although treatment with sodium chloride increased urine flow and electrolyte excretion, urine pH and urinary PGE_2 excretion remained practically unchanged. The excretion rate of PGE_2, however, increased by on the average 66% ($P < 0·05$) following treatment with sodium bicarbonate, which resulted in parallel rise in urine pH. In contrast, there was a significant fall in PGE_2 excretion and in urine pH following oral ammonium chloride. Urinary kallikrein excretion was not significantly altered by any of the three interventions mentioned above.

When values for PGE_2 excretion rates obtained in control periods were plotted against urine pH, urine or plasma osmolality and electrolyte excretion no correlation could be detected. For values obtained after oral fluid loads, urine pH was the only parameter showing a significant positive correlation to the excretion rate of PGE_2 ($r_S = 0·854$, $P < 0·001$) (Fig. 1).

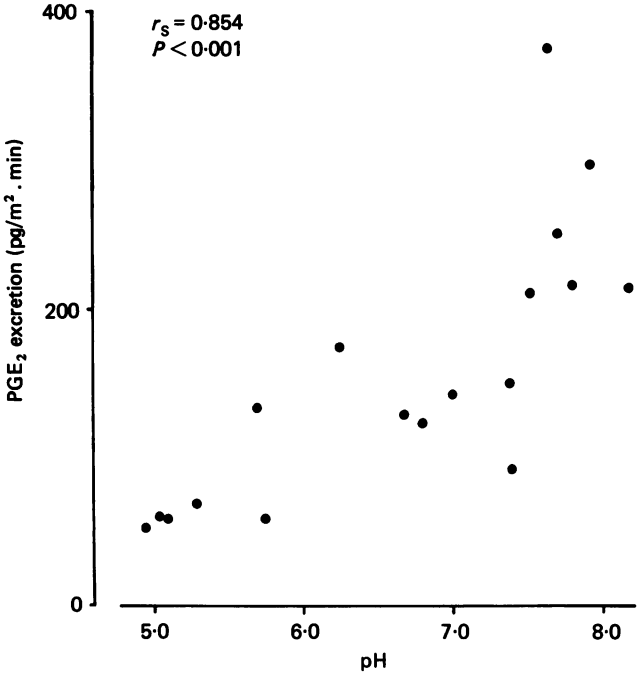

Fig. 1. Relationship between urine pH and PGE_2 excretion after oral sodium chloride, sodium bicarbonate and ammonium chloride loadings. r_S, Spearman's rank correlation coefficient.

DISCUSSION

PGE_2 in the urine is of renal origin, entering the tubular fluid in Henle's loop (Williams, Frölich, Nies & Oates, 1977). Although its urinary excretion rate has been commonly used as a non-invasive index of its renal synthesis, the role of urine flow and urine pH in regulation of PGE_2 excretion by conscious dogs is not fully understood. Therefore, the present study was directed toward an exploration of the relationship of PGE_2 excretion, urine flow and urine pH in conscious dogs. In order to distinguish between possible simultaneous effects of urine flow and pH, we investigated the effects of water deprivation and rehydration on PGE_2 excretion under conditions that had no significant effects on urine pH. On the other hand, we measured urinary PGE_2 excretion under conditions that produced maximal changes in urine pH with similar changes in water and electrolyte excretion.

In the present experiments conducted during water deprivation and after rehydration PGE_2 excretion decreased parallel with urine flow at constant urine pH, confirming earlier results with respect to flow dependency of urinary prostaglandin excretion in conscious dogs (Kirschenbaum & Serros, 1980; Wright *et al.* 1981; Fejes-Tóth *et al.* 1983*a*) and in man (Kaye *et al.* 1980; Walker *et al.* 1981; Haylor *et al.* 1986*a*). On the other hand, flow-independent (Fejes-Tóth, Náray-Fejes-Tóth, Rigter & Frölich, 1983*b*) as well as flow-dependent (Haylor & Lote, 1986*b*) PGE_2 excretion has been reported in the rat. Whether this discrepancy might be attributed to differences in the range of urine flows studied or other differences in experimental

conditions (e.g. conscious *versus* anaesthetized animals) is presently not clear. Supporting published reports (Zucker, Nasjletti & Schneider, 1983), the present study also demonstrates dependency of urinary PGE_2 excretion on the state of hydration regardless of the mechanism(s) involved in governing the excretion rate of PGE_2.

Our results also demonstrate that – as reported in rats (Haylor *et al.* 1984) and man (Haylor *et al.* 1986*a*) – urine pH can be a determinant of urinary PGE_2 excretion in the conscious dog, the excretion rate of PGE_2 being higher in alkaline than in acidic urine. Flow-dependent components seem unlikely to contribute to these changes as oral treatment with sodium chloride caused similar increase in urine flow, but did not alter PGE_2 excretion and urine pH. The present results, however, offer no information regarding the mechanism(s) by which urine pH might affect renal PGE_2 excretion. The following possibilities should be considered. First, stability of PGE_2 excreted may be affected by changes in urine pH. This explanation is, however, unlikely since samples were immediately acidified after the end of urine collection periods, and thus initial conditions were identical in each sample for PGE_2 determination. Secondly, the ionization of PGE_2 present in the tubular fluid and therefore its lipid solubility may be determined by pH of tubular fluid as it was suggested by Haylor *et al.* (1984). Although there is no direct experimental evidence supporting this explanation, a previous report showing pH-dependent excretion of indolylacetic acid, a weak acid, (Milne, Crawford, Girao & Loughbridge, 1960) makes it very possible. Thirdly, one should also consider the possibility that tubular – or more likely intracellular – pH could modify synthesis and/or metabolism of PGE_2.

Kallikrein is known to be synthesized within the kidney and to be regulated by hormones that control renal function (Margolius, 1984). However, little is known about the role of urine flow and pH in regulation of its excretion. There are reports that kallikrein excretion is not affected by water depletion or by subsequent rehydration in dogs (Zucker *et al.* 1983) or by water loading in humans (Margolius, Horwitz, Geller, Alexander, Gill, Pisano & Keiser, 1974). The present observations that kallikrein excretion was similar in water-restricted and water-loaded dogs give further support to the notion that kallikrein excretion is independent of the state of hydration. Furthermore, in the present experiments we could not detect a relationship between urinary kallikrein excretion and urine pH. In contrast, increased kallikrein excretion has been reported in rats when urine was made acidic (Scicli, Diaz & Carretero, 1983). This discrepancy might be attributed to species differences or to differences in measurements of kallikrein activity. Scicli *et al.* (1983) have measured kallikrein activity by its kininogenase activity under conditions when urinary peptidases other than kallikrein (e.g. urinary serine protease) are active (Hial, Keiser & Pisano, 1976), and thus these peptidases could artifactually alter the measured levels of kinins (Margolius, 1984). Nevertheless, one should remember that urinary kallikrein is not the only factor reflecting changes in the activity of the intrarenal kallikrein–kinin system. Thus the present study does not exclude the possibility that the state of hydration and/or urine pH might influence the other components of this system. The present data suggest that urinary kallikrein and PGE_2 excretion are not closely interrelated or interlinked.

In conclusion, the present findings give further support to the concept of pH

dependency of urinary prostaglandin excretion by demonstrating that urine pH besides urine flow rate might be an important determinant of PGE_2 but not of kallikrein excretion in the conscious dog. These results also call attention to the importance of measuring urine pH in experiments where prostaglandin excretion is determined.

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