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EXPERIMENTAL DISSOCIATION OF THE EFFECTS OF PROSTAGLANDINS ON RENAL SODIUM AND WATER REABSORPTION BY CYCLO-OXYGENASE INHIBITORS IN THE RAT

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1 The relative importance of the effect of prostaglandins on renal sodium and water reabsorption was assessed in rats.

2 Clearance experiments were performed on 24 anaesthetized rats divided into 3 groups. Each group was infused throughout either with Ringer solution at 9 ml/h (Protocol I), or at 3 ml/h (Protocol II) or with hypotonic fluid at 5 ml/h (Protocol III). Clearance periods were performed before and after intravenous injection of indomethacin (5 mg/kg) and then of aspirin (20 mg/kg). The natriuretic response to different degrees of volume expansion was not modified during the action of the inhibitors.

3 When baseline urine osmolality (Uosm) was high (Protocol II) no further increase occurred in the presence of prostaglandin inhibition. Conversely, Uosm rose from 771 ± 134 to 1356 ± 414 and from 575 ± 245 to 841 ± 407 mosm/kg ($P < 0.05$) in Protocol I and Protocol III respectively, when antidiuretic hormone secretion was inhibited by the higher degree of volume expansion.

4 There was a significant correlation between the change in urine flow rate induced by cyclo-oxygenase inhibitors and the attendant variations in Na excretion, $r = 0.42$, $n = 41$, $P < 0.01$.

5 Thus, prostaglandins affect Na loss during saline load as a side effect of their action on water permeability. They could play an important role in volume depletion by counterbalancing the large secretion rate of renal vasoconstrictors.

Introduction

The mechanism underlying the intrarenal action of prostaglandins in controlling Na excretion is uncertain (Lee, Patak & Mookerjee, 1976). The effect could be either direct or mediated by the impaired response of the distal nephron to antidiuretic hormone (ADH) in the presence of prostaglandin (Anderson, Berl, McDonald & Schrier, 1975): due to the phenomenon of flow-dependence of Na excretion (Coleman, Arias, Carte, Rector & Seldin, 1966; Bartoli, Molaschi, Matone, Viara & Pupita, 1966) urinary Na will follow the changes in urine flow rate induced by cyclo-oxygenase inhibitors. Furthermore, the effect could be secondary to the action of prostaglandin on renin release (Vinci, Gill, Bowden, Pisano, Izzo, Rodfor, Taylor, Zusman, Bartter & Keiser, 1978). As several studies on man, in health and disease, failed to demonstrate a normal role of prostaglandin in controlling Na and water metabolism, (Bartoli, Arras, Satta, Soggia, Faedda & Olmeo, 1978a; Bartoli, Faedda, Soggia, Satta, Olmeo & Pilia, 1978b; Bartoli, Arras, Faedda, Soggia & Satta, 1979; Bartoli, Arras, Faedda, Soggia, Satta & Olmeo, 1980) we attempted in the present study to distinguish between direct and indirect effects on animals under controlled experimental conditions.

Methods

Experiments were performed on 24 rats weighing 200 to 300 g. After 12 h without food, the animals were anaesthetized with phenobarbitone (100 mg/kg) and placed on a heated surgical table.

The left kidney was exposed through a flank incision and the trachea, jugular vein, femoral artery and left ureter were cannulated with polyethylene tubing.

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After a priming dose of 3 ml of 0.9% w/v NaCl solution (saline) to replace surgical losses, the animals were continuously infused with fluids containing ^{131}I-Hypaque for clearance measurements. Complete urine collections were performed in calibrated glass capillaries; blood was sampled via the femoral artery and its losses quantitatively replaced.

The animals were studied during saline loading by continuous infusion of a Ringer solution at 9 ml/h (Protocol I), 3 ml/h (Protocol II) and during the infusion of a hypotonic solution at 5 ml/h (Protocol III). The latter solution was obtained by diluting the Ringer with an equal volume of distilled water. When a steady state was reached, as indicated by constant urine flow rates, baseline clearance periods of 5 to 27 min duration were performed during continuous infusion of Ringer or hypotonic solution alone. They were repeated 15 min after the injection of indomethacin (Indo 5 mg/kg by i.v. injection) and again 15 min after the administration of aspirin (20 mg/kg i.v.), while the infusion of fluids was kept constant. In 50% of the animals the administration of aspirin preceded Indo. In urine (U) and plasma (P) samples Na and K were measured with a flame photometer, osmolality by an advanced osmometer and glomerular filtration rate (GFR), urine flow rate ($\dot{V}$), Na and K excretion calculated. Radioactive iodine was counted in a well-type scintillation counter.

The data were processed statistically (Snedecor & Cochran, 1972): means ± standard deviations, regression and correlation coefficients were calculated and their differences tested by variance analysis and, when appropriate, by paired Student's *t* test. More detailed descriptions of the techniques used have been published elsewhere (Bartoli & Earley, 1971).

Results

The results are shown in Table 1. Since the aim of our study was to examine the importance of prostaglandins in the renal response to saline infusion, the table shows results obtained in control conditions and those during maximum inhibition of prostaglandin biosynthesis, when both inhibitors were acting. The results obtained with each inhibitor alone are not detailed in the table. Urine flow rate divided by GFR ($\dot{V}$/GFR) fell significantly with the cyclo-oxygenase inhibitors during isotonic infusions (Protocols I and II). This was mainly due to a rise in GFR, while $\dot{V}$ did not change significantly in absolute terms.

Na excretion by the experimental kidney was proportional to the rate of infusion and remained stable in each Protocol irrespective of the presence of cyclo-oxygenase inhibitors. Urine osmolality showed the most interesting findings: it rose during cyclo-oxygenase inhibition in Protocols I and III whereas it remained stable in Protocol II. Thus, it appears that Uosm rises with cyclo-oxygenase blockade only when it is below the maximal value attainable during sustained volume expansion.

There were no differences in plasma electrolytes and osmolalities in the presence of inhibitors: plasma Na was 143 ± 8 mmol/l during control conditions as compared with 144 ± 8 mEq/l during cyclo-oxygenase blockade. There were no qualitative differences between the results obtained with either inhibitor alone, as compared to those recorded when both aspirin and Indo were acting. The paired *t* test performed between the cyclo-oxygenase inhibitors showed that the rise in Uosm was significantly more pronounced for Indo ($P < 0.05$ by paired analysis with respect to aspirin). A similar paired difference was also present for $\dot{V}$/GFR, which fell more markedly with Indo than with aspirin ($P < 0.05$).

Urinary K excretion was significantly though slightly increased by Indo in Protocol III, probably as a consequence of the rise in urine concentration, which would be expected to affect preferentially solutes that are excreted, rather than reabsorbed, by the distal tubule.

Discussion

The aim of the present paper was to assess the importance of prostaglandin in the natriuretic response to volume expansion and on ADH-dependent water reabsorption.

Volume expansion was obtained with different rates of infusion in Protocols I and II and with hypotonic fluids in Protocol III: in all Protocols natriuresis ensued, and the steady-state Na excretion was not modified by cyclo-oxygenase inhibition. Thus, prostaglandins are not essential in the renal response to saline infusion, and the inhibition of proximal reabsorption (Bartoli & Earley, 1971) is not controlled by them.

The importance of prostaglandins in water reabsorption can be examined with respect to the changes in baseline urine osmolality. In Protocol I urine osmolality was relatively low (711 ± 134 mosm/kg), due to inhibition of ADH release by the high degree of volume expansion: cyclo-oxygenase inhibitors caused Uosm to rise significantly at the expense of fractional urine flow rate, without affecting Na excretion. This effect was not apparent in Protocol II, where Uosm had reached during baseline conditions the maximum value attained in the presence of both Indo and aspirin in Protocol I. Presumably ADH suppression was less with the lower rate of saline infusion in Protocol II, and cyclo-oxygenase inhibitors could not enhance an already maximal ADH

Table 1 Effects of cyclo-oxygenase inhibition on renal function in anaesthetized rats

Protocol	GFR (μl/min) Control		PG-block	V̇/GFR × 10^3 Control		PG-block	UK·V̇ (μEq/min) Control	PG-block	UNa·V̇ Control	PG-block	Uosm (mosm/kg) Control		PG-block
I	546± 272	*	765± 269	46± 28	*	25± 9	1.3± 0.8	1.2± 0.8	5.4± 2.9	5.0± 2.1	771± 134	**	1356± 414
II	750± 159	*	1243± 503	13± 5	**	8± 4	1.2± 0.7	1.3± 0.5	2.1± 0.7	2.1± 1.1	1130± 289		1248± 340
III	808± 146		797± 272	24± 3		21± 15	1.0± 0.3	1.3± 0.6	2.4± 0.7	1.9± 1.2	575± 245	*	841± 407

Mean results ± s.d. obtained with different protocols during baseline conditions (Control) and during the action of both indomethacin and aspirin (PG-block). Although aspirin and indomethacin were given in sequence, the results obtained with either inhibitor alone are not shown, being qualitatively the same as those measured immediately afterwards, when both were present.
*$P < 0.05$; **$P < 0.01$ between adjacent measurements.

effect. Cyclo-oxygenase inhibitors had a maximum effect in Protocol III, when baseline urine osmolality was at the lowest level measured in the present experiments, though still above plasma. Therefore, it seems that aspirin and Indo can act by rendering low amounts of ADH as effective as higher secretion rates in promoting water reabsorption across distal tubular epithelium.

In agreement with this interpretation, cyclo-oxygenase inhibitors have no effect during maximal water diuresis in man (Bartoli *et al.*, 1978a, b) and animals (Work, Bachler, Kotchen, Talwalkar & Luke, 1980), while they produce a slight, non-progressive water retention in normal non diuresing subjects (Gullner & Bartter, 1979).

It is possible that this effect on water may transiently affect Na excretion, since the latter is flow-dependent (Bartoli *et al.*, 1966; Coleman *et al.*, 1966). This may have led some authors to postulate an independent effect of prostaglandins on Na excretion. Our study demonstrates that prostaglandin inhibitors display a clear effect on water without any influence on Na reabsorption. Figure 1 shows that, although there were no modifications in average Na excretion, the inhibitors caused changes in natriuresis in opposite directions: if these were random in nature, they would not be expected to correlate with the simultaneous variations in water excretion. The figure shows that this correlation was present and significant. Therefore, the direct effect of prostaglandins on Na transport reported by others (Lee *et al.*, 1976) may be a side-effect of the enhancement of ADH-mediated water reabsorption.

Finally, GFR was not impaired by Indo and aspirin in the volume expanded animals used in this study. This observation indicates that prostaglandins are not important vasodilators in this condition, in agreement with others (Schor, Ichikawa & Brenner, 1980) who showed an important effect of cyclo-oxygenase inhibition on renal haemodynamics during volume depletion, both in animals (Schor *et al.*, 1980) and patients (Boyer, Zia & Reynolds, 1979).

In conclusion, our study shows that the natriuresis attending different degrees of volume expansion is not modified by cyclo-oxygenase inhibitors, whilst they do enhance the effect of ADH on water excretion. The sudden change in urine flow rate caused by the inhibitors may transiently affect Na excretion in a way not dependent on a direct action on trans-epithelial transport.

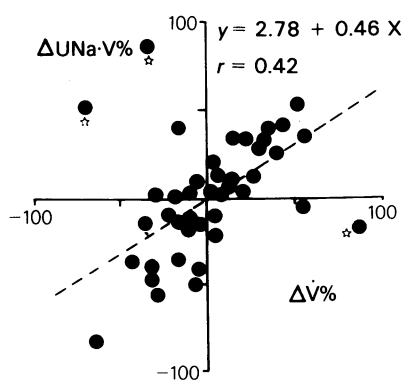

Figure 1 The changes measured in urine flow rate between two subsequent experimental conditions are shown on the abscissa scale as a percentage of the initial value. They are plotted against the simultaneous changes in Na excretion, shown on the ordinate scale. The correlation is significant ($P < 0.01$) even including three clearly aberrant points, marked by asterisks.

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