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RENAL FUNCTION IN CONSCIOUS RATS AFTER INDOMETHACIN. EVIDENCE FOR A TUBULAR ACTION OF ENDOGENOUS PROSTAGLANDINS

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SUMMARY

1. Conscious rats, with implanted carotid arterial cannulae, received a saline infusion (5·8 ml./hr) via a tail vein for a 6 hr period. The urinary excretion of water, sodium, potassium, urea and the osmolal output were monitored, together with the systemic blood pressure. Glomerular filtration rate (inulin clearance) and effective renal plasma flow (*p*-aminohippurate clearance) were also measured. Four hours after the start of the infusion, indomethacin (10 mg/kg body weight) in buffered saline, or buffered saline alone, was administered via the tail vein.

2. Following indomethacin administration, urine flow, sodium output and osmolal output were markedly reduced ($P < 0{\cdot}01$). However, there were no measurable changes in the systemic blood pressure, glomerular filtration rate, or effective renal plasma flow.

3. It is concluded that the changes in urinary excretion observed after indomethacin are not dependent on changes in effective renal plasma flow or glomerular filtration, and it is suggested that indomethacin inhibits the synthesis of endogenous prostaglandins which directly influence renal tubular function.

INTRODUCTION

Prostaglandins have both diuretic and natriuretic actions (Johnston, Herzog & Lauler, 1967; Fulgraff & Brandenbusch, 1974; Fine & Trizna, 1977), but the importance of endogenous prostaglandins in determining or regulating renal solute and water excretion remains uncertain. Following the *in vivo* administration of prostaglandin synthetase inhibitors, the renal excretion of sodium or water or both has been reported to be: (*a*) increased in conscious dogs (Kirschenbaum & Stein, 1976); (*b*) decreased in rats anaesthetized with Amytal (Leyssac, Christensen, Hill & Skinner, 1975) and dogs anaesthetized with pentobarbitone (Feigen, Klainer, Chapnick & Kadowitz, 1976) or (*c*) unaffected in conscious rats during water diuresis (Berl, Raz, Wald, Horowitz & Czaczkes, 1977). The inconsistent changes in solute and water excretion after prostaglandin synthetase inhibition may be explained by differences in the animal species used and by the variety of conditions under which experiments were performed, including the degree of volume expansion and the state of water balance and anaesthesia. For example expansion of blood volume (Papanicolaou, 1975), antidiuretic hormone (Kalisker & Dyer, 1972) and surgical stress under

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anaesthesia (Terragno, Terragno & McGiff, 1977) have all been shown to increase renal prostaglandin production. The administration of prostaglandin synthetase inhibitors to conscious human subjects, however, leads to decreases in solute and water excretion (Meiers & Wetzels, 1964; Haylor, 1980), and similar findings have been obtained with indomethacin in the conscious rat undergoing saline diuresis (Haylor & Lote, 1978).

McGiff & Malik (1976) have suggested that the changes in urinary excretion produced by prostaglandins are caused by a change in renal haemodynamics. In anaesthetized dogs, an increase in renal prostaglandin production leads to increased total renal blood flow and redistribution to the medulla (Chang, Splawinski, Oates & Nies, 1975), while inhibition of prostaglandin synthesis decreases renal blood flow (Lonigro, Itskovitz, Crowshaw & McGiff, 1973; Bailie, Barbour & Hook, 1975) and decreases its distribution to the medulla (e.g. McGiff, Crowshaw & Itskovitz, 1974; Kirschenbaum, White, Stein & Ferris, 1974). However, in conscious dogs, indomethacin has no effect on either the total renal blood flow (Swain, Heyndrickx, Boettcher & Vatner, 1975) or its distribution (Zins, 1975). A similar absence of effects on renal haemodynamics has been observed in conscious dogs with other prostaglandin synthetase inhibitors (Kirschenbaum & Stein, 1976). In this context, it has been shown recently that prostaglandin production by the kidney is elevated by surgery during anaesthesia (Terragno *et al.* 1977), possibly by activation of the renin–angiotensin system (Burger, Hopkins, Tulloch & Hollenburg, 1976).

It is likely therefore that conscious animals are more suitable experimental preparations than anaesthetized ones for the determination of the physiological role of endogenous prostaglandins in the regulation or control of renal function. Accordingly, we have performed experiments in the rat, using conditions which avoid stimulating renal prostaglandin production (i.e. conscious animals undergoing saliuresis with minimal volume expansion). We have determined the effects of indomethacin on urinary flow and composition, while monitoring systemic blood pressure. Clearances of inulin and *p*-aminohippurate were determined as measures of the glomerular filtration rate and effective renal plasma flow respectively.

METHODS

Experiments were performed on male rats (Sprague–Dawley strain, weight 250–400 g), which had been previously maintained on a rat-cake diet, with free access to water.

Preparation of animals. The rats were anaesthetized with Immobilon (Etorphine HCl and Methotrimeprazine; Reckitt and Colman) 0·05 ml. intraperitoneally (repeated as necessary) and a heparinized carotid arterial cannula was implanted. The cannula was passed subcutaneously to emerge dorsally from the neck. The animals were then allowed to recover after administration of Revivon (diprenorphine; Reckitt and Colman) and were used for experiment on the day after this operative procedure.

Experimental protocol. Each rat was lightly anaesthetized with ether, and a flexible cannula was implanted into a tail vein, as previously described (Lote & Snape, 1977). The animal was placed in a Perspex restraining cage and allowed to recover from the anaesthetic, during which time the carotid cannula was connected to a blood pressure transducer (Bell and Howell). Loading doses of inulin (30 mg) and *p*-aminohippurate (PAH) (30 mg) in 0·25 ml. 0·668% saline were administered via the tail vein. When recovery from the anaesthetic was complete, a constant infusion into the tail vein was begun, at a rate of 5·8 ml./hr. This infusion was con-

tinued for 6 hr. The composition of the infusate was: inulin, 1·8 g/100 ml.; PAH (sodium salt) 0·6 g/100 ml.; NaCl 0·668 g/100 ml., giving a sodium concentration of 140 mM. Urine samples were collected after 1 hr and thereafter at 30 min intervals throughout the 6 hr infusion period; voiding of urine was encouraged by gentle sensory stimulation. Four hours after the start of the infusion, animals received either indomethacin (10 mg/kg body wt.) in buffered saline or buffered saline alone (controls) via the tail vein, over a 15 min period, during which time the normal infusion rate of water, sodium chloride, inulin and PAH was maintained. The buffered saline was prepared according to Ganguli, Tobian, Azar & O'Donnell (1977) and consisted of 0·1 ml. 0·5 M-Na_2CO_3 (in which indomethacin was dissolved for the experimental series), 0·2 ml. 0·05 M-HCl, 0·4 ml. H_2O, 0·5 ml. 0·05 M-HCl in 0·9% sodium chloride. This was then diluted with saline containing inulin and PAH to maintain a constant concentration of sodium chloride, inulin and PAH in the infusate.

Blood samples (100 μl.) for the determination of inulin and PAH, were taken via the carotid cannula at 30 min intervals, beginning 2¼ hr after the start of the infusion.

Analytical procedures for urine and plasma. The volume of each urine sample was recorded, and urinary osmolality was determined by freezing point depression (Knauer cryostat unit). Urinary sodium and potassium were determined by flame photometry using a Beckman flame photometer with lithium internal standard. Urinary urea and ammonia were assayed by a modification of the method of Fawcett & Scott (1960). Inulin and PAH were determined in urine and plasma by modification of the methods of Bojesen (1952) and Smith, Finkelstein, Aliminosa, Crawford & Graber (1945) respectively; for both assays, plasma proteins were removed by precipitation as a zinc–protein complex using the method of Somogyi (1930).

Statistical analysis. Results are presented as mean ± S.E. of mean. Significance of differences *between* control and experimental group means was determined by Student's *t* test. The significance of differences *within* control and experimental groups was determined by the paired *t* test.

RESULTS

Systemic blood pressure

All the animals had a mean blood pressure within the range 100–140 mmHg, and there were no significant differences between the control (buffered saline) and experimental (indomethacin) series at any time during the experiments (Table 1).

In a preliminary series of experiments, in which indomethacin (10 mg/kg body wt.) was infused into the tail vein over a 2 min period, mean systemic blood pressure was increased by about 15 mmHg, for up to 30 min. Such an effect is not apparent when indomethacin is administered slowly (over a 15 min period), as in the present experiments.

Urinary composition

Urine samples were obtained 60 min after the start of the saline infusion, and thereafter at 30 min intervals for the remaining 5 hr of the experiment.

The time course of the changes in urinary excretion of water and sodium, the osmolal output and the urine osmolality, are shown in Fig. 1. The initial 2 hr of the infusion are regarded as an equilibration period, during which time the urine flow increased to match (and usually exceed) the infusion rate (i.e. 96·7 μl. min^{-1}) and the sodium output increased to match the sodium input (13·53 μmole min^{-1}). The relationship of urine flow to body fluid volume is considered below.

In the 2–4 hr period after the start of the infusion the urinary flow and excretion of solutes (sodium, potassium, urea, ammonia) and osmolal output were not significantly different between the control and experimental groups (Table 1).

The control group, in the 4–6 hr period showed no significant changes (compared

TABLE 1. Water and solute excretion in the 2–4 and 4–6 hr infusion periods. At 4 hr, the experimental series (denoted I) received indomethacin (10 mg/kg), and the control series (denoted C) received buffered saline. Results are mean ± S.E. of mean and both paired P- values (within groups) and unpaired P values (between groups) are included. For experimental series, $n = 8$; control series, $n = 7$; n.s., not significant.

		2–4 hr period	4–6 hr period	P for paired t test
Urine flow (μl. min^{-1})	C	108·0 ± 5·8	105·3 ± 4·7	n.s.
	I	109·8 ± 7·6	67·9 ± 8·2	$P < 0.002$
		n.s.	$P < 0.002$	
Osmolal output (μ-osmole min^{-1})	C	47·1 ± 2·8	46·6 ± 1·7	n.s.
	I	47·5 ± 2·1	34·7 ± 2·8	$P < 0.002$
		n.s.	$P < 0.01$	
Osmolality (μ-osmole ml.$^{-1}$)	C	445 ± 16	444 ± 11	n.s.
	I	438 ± 14	559 ± 60	n.s.
		n.s.	n.s.	
Potassium output (μmole min^{-1})	C	3·76 ± 0·44	2·87 ± 0·17	$P < 0.02$
	I	3·44 ± 0·13	2·49 ± 0·18	$P < 0.01$
		n.s.	n.s.	
Sodium output (μmole min^{-1})	C	13·67 ± 0·81	15·15 ± 0·71	n.s.
	I	13·50 ± 1·04	10·00 ± 1·23	$P < 0.01$
		n.s.	$P < 0.01$	
Urea output (μmole min^{-1})	C	11·04 ± 0·86	9·13 ± 0·69	$P < 0.001$
	I	10·13 ± 0·45	7·90 ± 0·45	$P < 0.01$
		n.s.	n.s.	
Mean systemic blood pressure (mmHg)	C	117 ± 6·6	115 ± 6·3	n.s.
	I	122 ± 2·8	117 ± 4·6	n.s.
		n.s.	n.s.	

to the 2–4 hr period) in urine flow, osmolal output, urine osmolality, or sodium output. However, the urea output decreased significantly ($P < 0.001$) as did potassium output ($P < 0.02$). Fig. 1 shows the 30 min values, and Table 1 the 2 hr values, for these variables.

The experimental group in the 4–6 hr period (i.e. following indomethacin administration) showed significant decreases in urine flow, sodium output and osmolal output, both in comparison with the 2–4 hr period paired t test) and the control series (t test), as shown in Table 1. The urea and potassium outputs also decreased in the 4–6 hr period, as they did in the control series.

The urine osmolality in the indomethacin treated animals showed a transient increase (Fig. 1) as the urine flow decreased, but this change was variable between individual animals, and was not statistically significant over the 2 hr period.

Body fluid volume

Because the urine flow did not instantaneously match the saline infusion rate (96·7 μl. min^{-1}), the animals became slightly volume-expanded in the initial 2 hr equilibration period. Subsequently, in the 2–4 hr period, urine flow in both series slightly exceeded the input so that by 4 hr, the mean change in body weight was less than 1% (control series 0·81 ± 0·36%; indomethacin series, 0·61 ± 0·34%). The

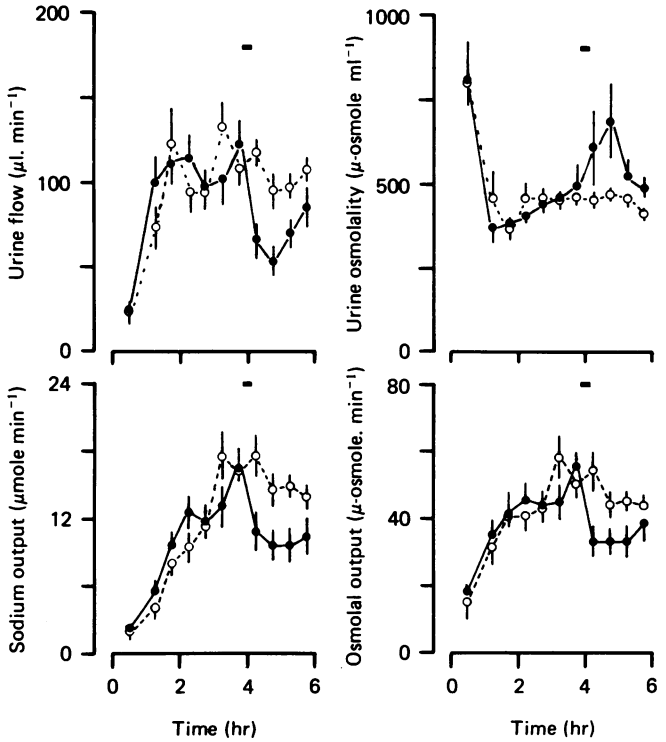

Fig. 1. Urinary flow, osmolality, sodium output and osmololal output. ●, experimental (indomethacin) series (n = 8); ○, control series (n = 7). The experimental series received indomethacin (10 mg/kg body weight) over 15 min (indicated by bar). Control series received buffered saline (vehicle) over the same period. Values plotted are mean ± S.E. of mean.

volume expansion for each rat was calculated at 30 min intervals as total fluid input minus total urine output, and the effects of indomethacin on body fluid volume are shown in Fig. 2. For each rat, the change in fluid balance from the 4 hr value (i.e. the time of indomethacin administration) was expressed as a percentage of the body weight. It can be seen that indomethacin leads to an increase in body fluid volume during the 4–6 hr period, the change being highly significant (Fig. 2) in comparison with the control group.

Glomerular filtration rate and effective renal plasma flow

Table 2 shows the renal clearance of inulin and PAH for the two series of experiments over the 2–4 and 4–6 hr periods. Neither the administration of indomethacin (experimental series) nor of buffered saline (control series) produced changes in glomerular filtration rate or effective renal plasma flow.

Urinary excretion and renal haemodynamics after indomethacin

Since some changes in renal solute excretion (notably for urea and potassium) occurred in the control series, we have attempted to clarify changes due solely to

TABLE 2. Renal clearance of inulin and PAH in the 2–4 and 4–6 hr infusion periods. At 4 hr, experimental series (I, $n = 8$) received indomethacin (10 mg/kg) and control series (C, $n = 7$) received buffered saline. Results are mean ± S.E. of mean. Significance of paired (within groups) and unpaired (between groups) t test is included; n.s., not significant.

		2–4 hr period	4–6 hr period	P for paired t test
PAH clearance	C	11·31 ± 0·95	11·21 ± 0·91	n.s.
(ml. min^{-1})	I	12·5 ± 0·63	12·3 ± 0·58	n.s.
		n.s.	n.s.	
Inulin clearance	C	3·38 ± 0·24	3·41 ± 0·20	n.s.
(ml. min^{-1})	I	4·03 ± 0·27	4·02 ± 0·25	n.s.
		n.s.	n.s.	

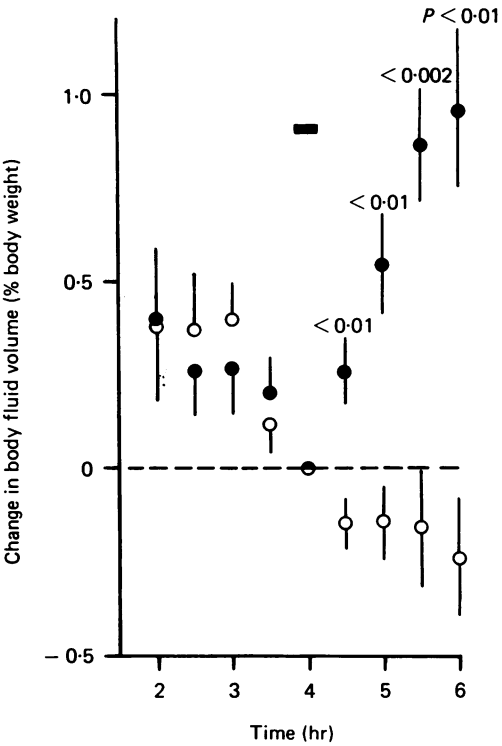

Fig. 2. Effect of indomethacin on body fluid volume. ●, experimental (indomethacin) series ($n = 8$); ○, control series ($n = 7$). Values are mean ± S.E. of mean. The change in body fluid volume from the 4 hr value is expressed as a percentage of the body weight. The P values are for the comparison between the experimental series and the control series (unpaired t test). Indomethacin (10 mg/kg) was administered to the experimental series over the period indicated by the bar.

indomethacin, by expressing the results obtained in the 2 hr period following treatment with either saline or indomethacin, as a percentage of the value in the 2 hr period before treatment (Fig. 3). The significant finding is that indomethacin is seen to reduce urine flow, osmolal output and sodium output, without changing the glomerular filtration rate or effective renal plasma flow.

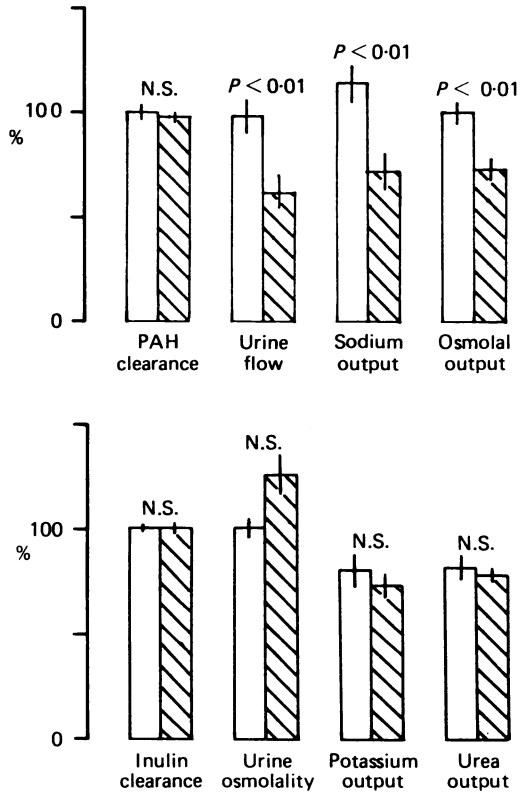

Fig. 3. Changes in urine flow and osmolality, sodium, potassium and urea outputs, osmolal output, and the clearance of inulin and PAH, in the 4–6 hr infusion period, expressed as a percentage of the 2–4 hr infusion period value. Hatched bars, experimental (indomethacin) series (n = 8), which received indomethacin at 4 hr. Open bars, control series (n = 7) which received buffered saline at 4 hr. Values are mean ± S.E. of mean. The P values refer to the significance of differences between control and experimental series (unpaired t test). N.S. = not significant.

DISCUSSION

The contention that the urinary changes induced by endogenous prostaglandins are due to altered renal haemodynamics is based largely on two sets of circumstantial evidence. First, a direct action of prostaglandins (of the E series) on tubular function was thought unlikely because in isolated high resistance epithelial tissues prostaglandins stimulate rather than inhibit active sodium transport (Barry & Hall, 1969; Lipson & Sharp, 1971). Secondly, a causal link was assumed because, in the anaesthetized animal, both haemodynamic and urinary changes are apparent following intravenous administration of prostaglandin E (Vander, 1968) or stimulation (Bolger, Eisner, Ramwell & Slotkoff, 1976) or inhibition (Aiken & Vane, 1973) of renal prostaglandin synthesis. However, in the anaesthetized dog the haemodynamic and urinary changes produced by exogenous prostaglandins have been separated. Although prostaglandin E_2 increases both renal blood flow and sodium excretion,

$PGF_{2\alpha}$ produces a natriuresis without increasing blood flow (Fulgraff & Brandenbush, 1974) while PGD_2 increases renal blood flow without influencing sodium excretion (Bolger, Eisner, Shea, Ramwell & Slotkoff, 1977). Feigen *et al.* (1976) have also demonstrated that following prostaglandin synthetase inhibition there is a difference in the time course of the reduction in total renal blood flow compared to the reduction in sodium output.

The present findings indicate that in the conscious rat, the effective renal plasma flow (clearance of *p*-aminohippurate), and therefore total renal blood flow, remain unchanged following prostaglandin synthetase inhibition with indomethacin. However, the renal excretion of water and sodium is reduced, without measurable changes in the mean systemic blood pressure or the glomerular filtration rate. The surgical procedure used to implant carotid cannulae did not contribute to the urinary changes following indomethacin treatment, because similar alterations in urinary excretion have been demonstrated in non-cannulated, conscious rats (Haylor & Lote, 1978); and the dose of indomethacin used (10 mg/kg) was the minimal dose required to produce a maximal inhibition of renal prostaglandin synthetase in the rat *in vivo* (Berl *et al.* 1977). McGiff & Malik (1976) suggested that although a haemodynamic explanation for the urinary changes produced by endogenous prostaglandins was the most likely, a direct action of prostaglandins on the renal tubule could not be discounted. The present results are consistent with the suggestion that in the conscious rat, the decrease in the renal excretion of water and sodium following indomethacin administration is a consequence of a reduction in the synthesis of prostaglandins involved in the control of tubular function.

Micropuncture evidence indicates that prostaglandin E_2 (Strandhoy, Ott, Schneider, Willis, Beck, Davis & Knox, 1974; Fulgraff & Meiforth, 1971), and prostaglandin synthetase inhibitors (Leyssac *et al.* 1975; Roman & Kauker, 1978) do not change the renal proximal tubular handling of sodium, so any direct tubular action of prostaglandin synthetase inhibition must occur at some more distal nephron site. It has been shown that prostaglandins have an effect on both the cortical and medullary regions of collecting tubule *in vitro* (Iino & Imai, 1978) and on the distal tubule (Fulgraff & Meiforth, 1971). Iino & Imai (1978) found that PGE_2 decreased the potential difference across cortical and medullary segments of isolated rabbit collecting tubules *in vitro*, and that this fall in potential difference was associated with a suppression of net sodium transport. The results were interpreted as indicating that PGE_2 inhibited sodium transport. However, although there is still some controversy concerning the effects of prostaglandins on the collecting tubule (e.g. Fine & Trizna, 1977; Stokes & Kokko, 1977), it should nevertheless be borne in mind that in transporting epithelia, potential difference is not an independent variable, since the potential difference across the tubule depends not only on sodium transport, but also on the conductance (ionic permeability) of the epithelium.

Leyssac *et al.* (1975) have pointed out that the collecting tubule is a 'high resistance' epithelium resembling frog skin and toad bladder, and in such epithelia, prostaglandins do not generally inhibit sodium transport. In fact, in frog skin prostaglandin E_1 stimulates active sodium transport; however, it also increases the permeability of the epithelium to sodium, potassium, calcium and chloride (Lote, Rider & Thomas, 1974). Prostaglandin synthetase inhibition, in addition to inhibiting sodium transport, also

increases the DC resistance (i.e. reduced the permeability) in the epithelium (Haylor & Lote, 1976, 1977). The findings reported in the present paper could be explained in terms of similar permeability changes occurring in the collecting tubule, i.e. a reduction in collecting duct permeability induced by prostaglandin synthetase inhibition with indomethacin, will reduce the back-flux of sodium from medullary interstitium to lumen. Conversely the natriuretic effect of prostaglandins could be accounted for by increased sodium back-flux from interstitium to lumen, down the concentration gradient. Evidence implicating the collecting duct as the site for the antidiuretic action of indomethacin has been obtained by Roman & Kauker (1978) using micropuncture studies in rats.

It has been suggested that indomethacin may reduce urine flow by reducing the synthesis of prostaglandins which antagonize ADH (Grantham & Orloff, 1968; Berl *et al.* 1977). However, in isolated epithelia the actions of prostaglandins are not identical to those of ADH (Lote *et al.* 1974) and although in the present experiments an increase in urine osmolality was frequently seen following indomethacin, there was no change in free water clearance. Nevertheless, it has been shown that in the rat, dog and man, indomethacin potentiates the hydro-osmotic effect of ADH on the renal tubule (Berl *et al.* 1977; Anderson, Berl, McDonald & Schrier, 1975; Lum, Aisenbury, Dunn, Berl, Schrier & McDonald, 1977). We regard these findings as support for the contention that prostaglandins exert physiological effects at the renal tubular level.

In some circumstances, indomethacin may elicit responses which are not related to prostaglandin synthetase inhibition; specifically it has been shown to inhibit phosphodiesterase activity *in vitro* (Flores & Sharp, 1972). However, in the rat, doses of indomethacin which inhibit renal medullary prostaglandin synthesis do not inhibit phosphodiesterase activity (Lum *et al.* 1977), and doses of aspirin which do not alter phosphodiesterase activity have effects identical to those of indomethacin on renal function (Berl *et al.* 1977).

Finally, the present results may give some indication of the involvement of prostaglandins in the renal response to volume expansion. Dusing, Melder & Kramer (1976) have suggested that prostaglandins help to mediate the renal response to volume expansion, and if this were the case, we might expect a correlation between the degree of volume expansion before indomethacin and the extent of sodium retention afterwards. However, a similar degree of sodium retention was seen in all the animals, regardless of the state of volume expansion at the time of indomethacin administration. Nevertheless, indomethacin led to a prolonged reduction in urine flow and sodium excretion to a level below that of the infusion rate, so that the animals became progressively volume-expanded, and this does suggest that prostaglandin synthesis is necessary to excrete a saline load effectively. It seems likely on the basis of the present findings, and our previous observations in isolated epithelia, that prostaglandin synthetase inhibition reduces sodium and water excretion by a tubular action, possibly by reducing the permeability of a distal nephron segment.

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