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The influence of prostaglandin E_2 and indomethacin on the renal corticomedullary solute gradient in the rat

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The renal corticomedullary solute gradient and the urinary excretion of fluid and solute were determined during a saline infusion in anaesthetized and conscious rats. In the anaesthetized group the influence of PGE_2 (100 ng min^{-1}) given by a local renal infusion was investigated. In the conscious group (where prostaglandin synthesis does not contribute to renal blood flow) the effect of indomethacin (i.v. 10 mg kg^{-1}) was determined. Following PGE_2 infusion the corticomedullary gradient for sodium was depressed, equalizing the sodium concentration between the papilla tip and the final urine. Sodium output and urine flow were elevated, but both urinary and gradient changes could be dissociated from any increase in renal blood flow, assessed by PAH clearance. Following treatment with the prostaglandin synthetase inhibitor indomethacin in the conscious rat, the corticomedullary osmotic gradient was elevated while the urine flow was reduced. This increase in the gradient produced by indomethacin was, however, found to be independent of the decrease in urine flow. The results of the present experiments are consistent with the hypothesis that prostaglandin synthesis helps to determine the renal corticomedullary osmotic gradient, and does so by a mechanism unlikely to involve changes in renal blood flow or tubular flow rate: a permeability hypothesis is proposed.

Although the major renal prostaglandin (PGE_2) has vasodepressor, vasodilator and natriuretic properties (Vander 1968), evidence is now accumulating which suggests that the synthesis of PGE_2 in the renal medulla does not play a major role in the control of renal haemodynamics (Zins 1975; Whorton et al 1978) or the long-term regulation of extracellular fluid volume (Hesse et al 1979; Tan et al 1980). In the renal medulla prostaglandin synthetase appears to be confined to two cell types, the interstitial cells (Zusman & Keiser 1977), and the cells of the collecting duct (Janszen & Nugteren 1971; Bohman 1977) and since prostaglandins in general are local hormones, it seemed worthwhile to investigate whether they may have a role in the control of medullary function.

The capacity of the kidney to synthesize prostaglandins increases from the cortex through the medulla to the papilla, in parallel with the increasing solute concentration (Larssen & Anggard 1973). Furthermore, Ganguli et al (1977), in the pentobarbitone anaesthetized rat, have shown that the papillary sodium chloride concentration is increased following the administration of the cyclo-oxygenase inhibitor indomethacin. This raises the possibility that products of cyclo-oxygenase metabolism could play a regulatory role in the maintenance of the corticomedullary solute gradient. Such a regulatory role could also involve a negative feedback step since hypertonic sodium chloride stimulates prostaglandin production in rat renal papillary homogenates (Danon et al 1978). Thus changes in the solute concentration gradient may be minimized by parallel changes in PGE production, and the present experiments were performed to further examine the relationship between prostaglandin synthesis and papillary osmolality.

The first experiment was to determine the effect of PGE_2 on the corticomedullary gradient for sodium, and its relation to the composition of the urine. PGE_2 was delivered locally to the kidney in the anaesthetized, laparotomized rat to avoid complications from changes in the systemic circulation. This was an essential experiment to perform since a variety of atypical renal responses to prostaglandins have been described in the rat (Malik & McGiff 1975; Weber et al 1975; Gerber & Nies 1979), making the interpretation of experiments with cyclo-oxygenase inhibitors difficult. Also, renal blood flow was monitored to ascertain whether a vascular mechanism could explain any changes in the gradient which were produced.

In a second experiment, confirmation was sought that the cortico-medullary osmotic gradient increases following the administration of the cyclo-oxygenase inhibitor indomethacin, in another

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experimental model. The conscious restrained rat was used since in this model renal blood flow is not maintained by prostaglandin production (Haylor & Lote 1980a). However, indomethacin does reduce urine flow in conscious rats and changes in urine flow per se, reflecting altered collecting duct flow, can modify the concentration gradient (Lote & Snape 1977). Therefore the relationship between the gradient and the urinary changes produced by indomethacin was investigated to establish whether any causal link could be established.

METHODS

Male rats (Sprague-Dawley strain, 250–400 g) were used. They had been maintained on a standard rat cake diet with free access to water.

Experimental protocol in anaesthetized rats

Anaesthesia was induced with pentobarbitone (60 mg kg^{-1} i.p.) and maintained by pentobarbitone (3 mg intravenously) every 45–60 min. Body temperature was maintained at 37 °C. A tracheostomy was performed and cannulae were placed in the left carotid artery (to record blood pressure) and in the right jugular vein. At time 0 loading doses of inulin 30 mg and *p*-aminohippurate (PAH) sodium salt 30 mg in 0·25 ml sodium chloride (0·153 M) were administered via the jugular vein, followed by a continuous infusion of sodium chloride 0·153 M at 50 μl min^{-1} together with inulin 3% and PAH 0·8% for the determination of the glomerular filtration rate and effective renal plasma flow respectively. A mid-line abdominal incision was made, both ureters were cannulated and a narrow bore cannula (external diameter 0·61 mm) was introduced into the left femoral artery and advanced up the aorta to a point just above the origin of the left renal artery but below that of the right renal artery. A continuous infusion of saline 0·153 M was maintained through this cannula at 10 μl min^{-1}.

Following a 3 h equilibration period, blood and urine samples were collected during a 2 h experimental period in the second hour of which either PGE_2 (100 ng min^{-1}) in a final concentration of 0·7% ethanol, or 0·7% ethanol alone was administered together with the aortic infusion. At the end of the experimental period, at the time of maximal change in urine formation, the kidneys were rapidly excised and immediately frozen in liquid nitrogen.

In kidneys removed from rats receiving prostaglandin E_2, tissue samples from both kidneys were assayed for sodium alone.

Experimental protocol in conscious rats

After implantation of a flexible tail-vein cannula under ether anaesthesia, each rat was secured in a cylindrical Perspex restraining cage; when the animal had fully recovered consciousness, an isotonic (0·153 M) sodium chloride infusion was begun, via the tail vein. The rate of infusion was 96·7 μl min^{-1}. This infusion continued for 5 h. After 3 h 52·5 min, the experimental group (n = 16) received indomethacin (10 mg kg^{-1}) over 15 min, via the tail vein. The indomethacin was made up in buffered saline vehicle as previously described (Haylor & Lote 1980a) and the infusate remained isotonic. A control group (n = 13) received only the vehicle. Urine samples were obtained at 30 min intervals. After 5 h the rats were anaesthetized with ether, and the kidneys were rapidly removed and frozen in liquid nitrogen. Tissue and urine samples were assayed as described below.

Slices from one kidney were used for the determination of renal tissue water, sodium and potassium, and slices from the other kidney for the determination of tissue urea and ammonia, as previously described (Lote & Snape 1977). Tissue osmolality was calculated as 2 ($Na^+ + K^+ + NH_4^+$) + urea (Levitin et al 1962). In addition to determining solute concentrations in the individual tissue slice, we have also calculated the whole papilla solute concentrations using data obtained from the papilla tip and papilla base tissue sections.

Tissue analysis

Six serial slices were cut from each kidney, from papilla tip, papilla base, inner medulla, outer medulla, inner cortex, and outer cortex (Atherton et al 1968). Each section was sealed in a previously weighed aluminium foil envelope, to limit the evaporation of water, and each envelope (and contents) was then weighed using a Beckman LM500 microbalance.

Analytical procedures

Inulin and PAH were determined in urine and plasma by modification of the methods of Bojesen (1952) and Smith et al (1945) respectively: for both assays plasma proteins were removed by precipitation as a zinc-protein complex using the method of Somogyi (1930). Urine osmolality was determined by freezing point depression (Knauer osmometer). Sodium and potassium were determined by flame photometry (Beckman: Klina flame photometer) and urea and ammonia were assayed by a modification of the method of Fawcett & Scott (1960).

Statistical procedures. Comparisons were made between results obtained from different animals using Student's *t*-test. For values obtained within the same animals the paired *t*-test was employed.

RESULTS

Effect of prostaglandin E_2 on renal sodium content and renal function

In rats infused with PGE_2 (100 ng min^{-1} i.a.) the sodium concentration in both papillary and medullary sections of the left kidney was significantly lower ($P < 0{\cdot}05$ n = 6) than in a control series receiving the infusion vehicle alone (n = 6) see Fig. 1(a). The cortical sodium concentrations remained unchanged. In rats infused with PGE_2, the sodium concentration in the whole papilla of the left kidney (220 ± 30 μmol ml^{-1} tissue H_2O) which received PGE_2 locally was also significantly lower ($P < 0{\cdot}05$ n = 6) than in the right kidney (363 ± 49 μmol ml^{-1} tissue H_2O) which acted as a contralateral control. The influence of PGE_2 on the degree of equilibration of sodium between the papilla tip and the final urine is shown in Fig. 1(b). PGE_2 infusion markedly reduced the difference between the papilla tip and urinary sodium concentrations in the locally infused kidney compared with the contra-control.

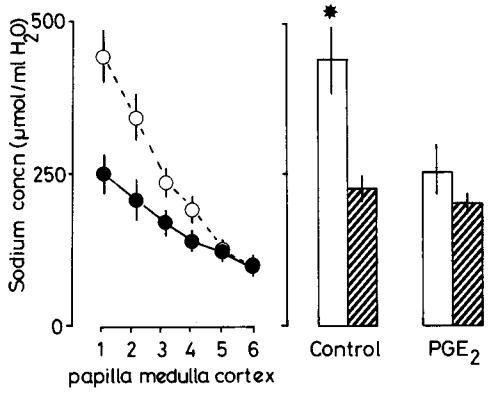

FIG. 1. Effect of PGE_2 (i.a. 100 ng min^{-1}) on (a) sodium concentration of renal tissue slices from the locally infused kidney (●) compared with the contralateral control (○) n = 6 and on (b) sodium equilibration between the papilla tip (open columns) and the final urine (hatched columns) using the contralateral kidney as the control. Vertical bars represent the s.e.m. $^*P < 0{\cdot}05$.

Before the administration of PGE_2 there was no significant difference between the function of the left kidney and its contralateral control. Following a transient decrease in systemic arterial blood pressure of up to 15 mmHg which lasted for 5–10 min, PGE_2 (100 ng min^{-1} i.a.) produced the following changes in the function of the left kidney compared with its contralateral control measured in the last 30 min of the infusion. Both the urine flow (20·6 ± 3·5 compared with 8·02 ± 1·2 μl min^{-1}) and the sodium output (3·84 ± 0·66 compared with 1·77 ± 0·34 μmol min^{-1}) were significantly higher ($P < 0{\cdot}02$) while the urine osmolality was significantly lower (844 ± 99 compared with 1562 ± 169 m osmol kg^{-1} H_2O) on the infused side ($P < 0{\cdot}01$). Neither inulin clearance (1·27 ± 0·11 compared to 1·42 ± 0·11 ml min^{-1}) nor potassium output (1·18 ± 0·16 compared with 1·12 ± 0·04 μmol min^{-1}) showed any significant change. Also, in spite of the large changes in the gradient and urine flow during PGE_2 infusion, the PAH clearance on the left infused kidney (3·79 ± 0·32 ml min^{-1}) was not significantly different from the contralateral control (3·67 ± 0·23 ml min^{-1}). The influence of PGE_2 on renal function is summarized in Fig. 2. However, the PAH clearance values of both kidneys were (unlike inulin clearance) significantly higher during the last 30 min period of the PGE_2 infusion ($P < 0{\cdot}05$) than during the 30 min period preceding the infusion where values of 3·42 ± 0·25 ml min^{-1} (E_2-infused) and 3·37 ± 0·20 ml min^{-1} (contralateral control) were obtained. In a separate series of experiments where rats received the infusion vehicle alone PAH clearance in both the infused kidney and its contralateral control remained unaltered.

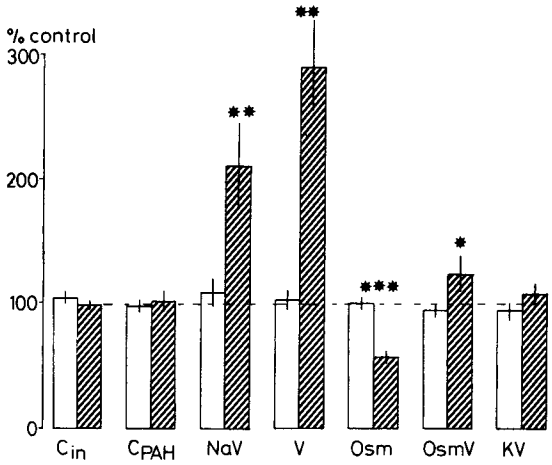

FIG. 2. Effect of PGE_2 (i.a. 100 ng min^{-1}) on renal function of both the locally infused (hatched columns) and the contralateral control kidneys (open columns) n = 6. Measurements taken in the 30–60 min of the infusion are expressed as a percentage of the value obtained in the 30 min before the infusion started for: inulin clearance (C_{in}), PAH clearance (C_{PAH}), sodium output (NaV), urine flow (V) urine osmolality (Osm), osmolal output (OsmV) and potassium output (KV). $^*P < 0{\cdot}05$, $^{**}P < 0{\cdot}02$, $^{***}P < 0{\cdot}01$.

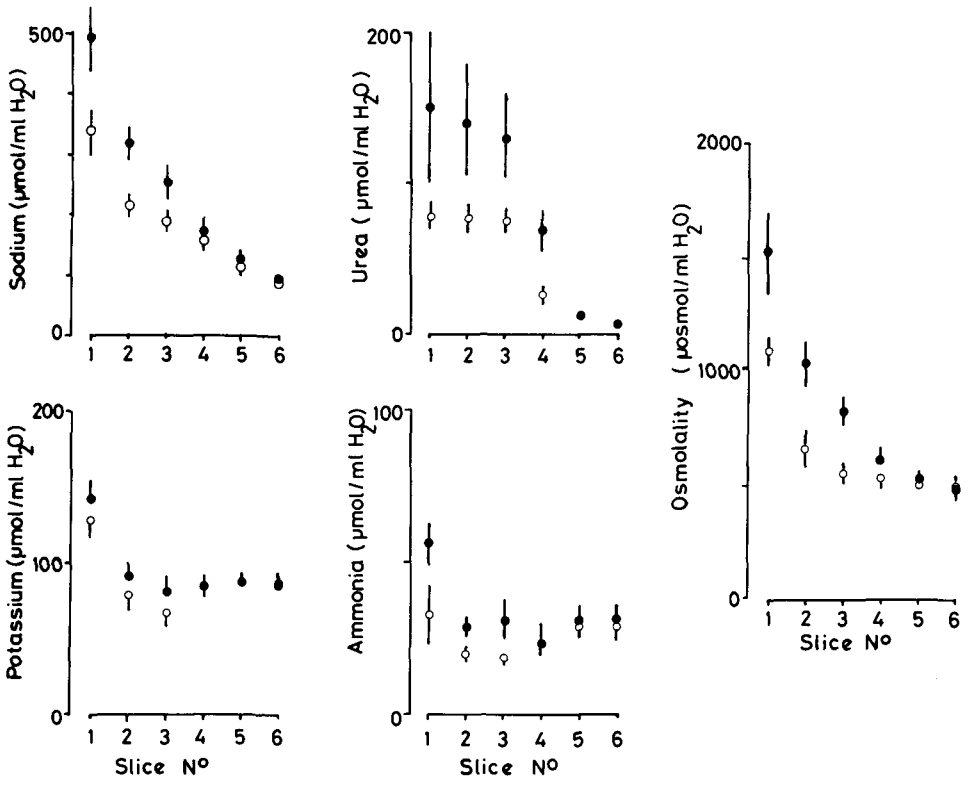

FIG. 3. Renal tissue solute concentration gradients in the control series (○ n = 13), and the indomethacin-treated series (● n = 16). The slice numbers are the tip and base, respectively of the following renal sections: papilla (1–2), medulla (3–4), cortex (5–6). Values given are mean ± s.e.m. Tissue osmolality was calculated as $2(Na^+ + K^+ + NH_4^+)$ + urea.

Effect of indomethacin on corticomedullary solute gradient and correlation to change in urine flow

Fig. 3 shows the renal tissue solute concentration gradients in indomethacin-treated (n = 16) and control animals (n = 13). In animals treated with indomethacin the whole papilla sodium concentration (389 ± 28·1 μmol ml^{-1} tissue H_2O) was higher ($P < 0.001$) than that of control rats (242 ± 11·3 μmol ml^{-1} tissue H_2O), as was the tissue osmolality (indomethacin group 1187 ± 78, control group 701 ± 78 μosmol ml^{-1} tissue H_2O). The mean whole-papilla urea concentration in the indomethacin-treated animals (121·7 ± 22·5 μmol ml^{-1} tissue H_2O) was higher than that of the control group (69·3 ± 8·4 μmol ml^{-1} tissue H_2O), but the urea concentration showed great variability between individual animals, and the difference between the two groups was not significant.

Following the administration of indomethacin the urine flow fell from 103·9 ± 7·3 to 87·3 ± 8·0 μl min^{-1} (n = 16 $P < 0.05$). However, although some individual animals failed to show a change in urine flow after indomethacin, in these rats the papillary tissue osmolality was still elevated. When the relationship between the urine flow and papillary tissue osmolality in each rat was assessed following indomethacin treatment, a highly insignificant correlation (r = 0·1065 $P > 0.4$) was found. The independence of the increased papillary osmolality from any simultaneous decrease in urine flow is described in Fig. 4 where animals were divided (by a ranking procedure) into two groups based on whether indomethacin produced either a significant decrease (69·1 ± 8·2 μl min^{-1} n = 8) or no change (105 ± 7·1 μl min^{-1} n = 8) in urine flow. In both groups however, the whole papillary tissue osmolality was still significantly higher ($P < 0.05$) than in the control series.

DISCUSSION

The finding that the osmolality of both medullary and papillary tissue segments increases following the

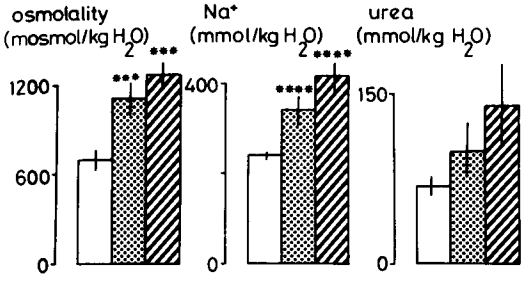

FIG. 4. Renal papillary tissue solute concentrations after 5 h saline infusion. Control group (open columns n = 13); indomethacin treated animals in which there was no change in urine flow (stippled columns n = 8); indomethacin-treated animals in which urine flow decreased (hatched columns n = 8). *P* values refer to differences between experimental and control groups (unpaired *t*-test).

administration of an inhibitor of prostaglandin biosynthesis (indomethacin) supports the possibility that the corticomedullary osmotic gradient could be maintained in part by the synthesis of products of renal cyclo-oxygenase. Since the major product of arachidonic acid in the renal medulla is PGE_2, for this interpretation to be possible PGE_2 should at least be able to decrease the concentration gradient. However, in the rat, prostaglandins have been reported to produce a variety of responses on renal blood flow (Malik & McGiff 1975; Baer & McGiff 1979) and urine formation (Weber et al 1975) which are atypical compared with other species, and such responses would be much more likely to result in an increase rather than a decrease in the osmotic gradient. In the present experiments, when PGE_2 was delivered locally to the kidney the corticomedullary gradient was markedly depressed. The tissue analysis was performed at the time of maximal increase in sodium output and urine flow and in this respect at least, the renal properties of PGE_2 in the rat were similar to those described for other species. The depression of the gradient occurred in the absence of altered systemic blood pressure or an increase in PAH clearance when compared with the contralateral control, making a vascular explanation an unlikely one for the changes seen. Prostaglandins can alter the intrarenal distribution of blood flow but such effects have never been described in the absence of changes in total renal blood flow. In the present experiments, the gradient changes were unrelated to altered PAH clearance, although PAH clearance did increase following treatment with PGE_2 *both* in the locally infused and the contralateral control kidneys, by some 10%. Recently, we have been able to describe full renal vasodilator responses to PGE_2 in the rat using an electromagnetic flow probe and retro-peritoneal surgery (Haylor & Towers 1982).

In the rat, inhibition of prostaglandin synthesis is therefore a plausible explanation for the increase in medullary osmolality seen with indomethacin. The dose used, 10 mg kg^{-1} is the minimum dose required to produce maximum inhibition in the rat renal medulla as described by Berl et al (1977). Like PGE_2, the effect of indomethacin could not be attributed to changes in total renal blood flow since in the conscious restrained rat it does not alter PAH clearance (Haylor & Lote 1980a). The lack of effect of indomethacin on total renal blood flow has also been well described in the conscious dog (Zins 1975). The mechanism of the gradient effects for PGE_2 could have been due to an increased tubular flow rate since they occurred in the presence of a 250% increase in urine flow and changes in urine flow reflecting altered collecting duct flow can modify the concentration gradient (Lote & Snape 1977). However, such an explanation is perhaps unlikely since it could not be applied to the changes associated with indomethacin where the papillary osmolality increased whether it was accompanied by a parallel decrease in urine flow or not. If neither changes in renal blood flow nor collecting duct flow are responsible, by what mechanism could PGE_2 depress, and indomethacin elevate, the renal corticomedullary osmotic gradient? Our findings with PGE_2 show similar changes to those described in the rat by Fulgraff & Meiforth (1971) but in our experiments the diuresis following PGE_2 was characterized by a high degree of equilibration of sodium between the papilla tip and the final urine. Our previous findings (Haylor & Lote 1980a) indicate that indomethacin can reduce sodium excretion without affecting potassium excretion in the conscious rat. It is unlikely that such an effect could be accounted for by an action of indomethacin on the loop of Henle, since, for indomethacin to increase the medullary solute concentration, it would have to stimulate NaCl extrusion from the ascending limb of the loop. This should then reduce the delivery of sodium to the sodium/potassium exchange sites in the distal tubule, and lead to a decreased potassium secretion, and hence to decreased potassium excretion. In the present experiments, no significant change in potassium excretion was seen in response to PGE_2 infusion. It seems probable then, that prostaglandin synthetase inhibition alters the corticomedullary solute gradient, and urinary sodium excretion, by an effect on the collecting ducts. The

collecting duct cells are known sites of prostaglandin synthetase activity (Cavallo 1976), and in experiments on isolated epithelia (frog skin), prostaglandins increase the ionic permeability (Lote et al 1974), and prostaglandin synthetase inhibition reduces the conductance (Haylor & Lote 1976, 1977). Similar permeability changes occurring in the collecting ducts (which, like frog skin, are 'high resistance' epithelia—Leyssac et al 1975) could account for the effects of prostaglandin synthetase inhibition on urinary excretion, and on the corticomedullary solute gradient, by regulating the rate of backflux (of NaCl) from the medullary interstitium to the collecting ducts.

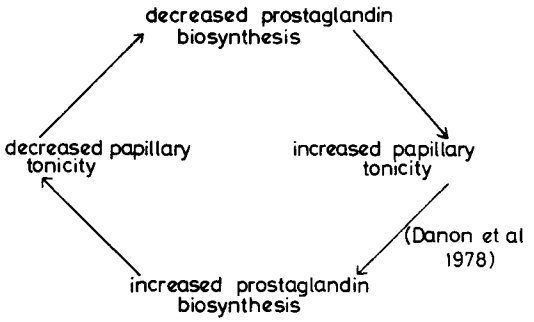

FIG. 5. Scheme illustrating proposed feedback loop whereby prostaglandin biosynthesis regulates papillary osmolality.

Direct evidence for an effect of prostaglandin E_2 (the main renal medullary prostaglandin) on the collecting duct has been obtained from experiments using rabbit isolated perfused collecting tubules. Iino & Imai (1978) found that PGE_2 suppressed net sodium transport out of the collecting ducts, but in these experiments it was not possible to determine whether this suppression was due to reduced active sodium transport, or to increased permeability of the ducts. In similar experiments Stokes & Kokko (1977) showed that PGE_2 inhibited efflux of sodium from the collecting tubule lumen, but did not affect the backflux, thereby suggesting an inhibition of active transport, and making the collecting duct an exception to the rule that prostaglandins stimulate sodium transport in high resistance epithelia (Leyssac et al 1975). However, there are major differences between the isolated, perfused collecting tubule, and the tubule in-vivo. In particular, in the experiments of Stokes & Kokko (1977) there was no gradient for backflux, and it therefore remains a possibility that small changes in permeability, which do not significantly change the backflux in-vitro, could do so under the influence of the high concentration gradient in-vivo.

It is thus apparent that prostaglandin synthetase inhibition increases the corticomedullary solute gradient, and that this effect, although it may *cause* changes in urine flow, is not *due* to such changes. The evidence from our experiments leads us to favour an explanation for these findings in terms of changes in collecting duct ionic permeability. It should be pointed out, however, that Stokes (1979), using isolated perfused segments of rabbit loops of Henle, has shown that exogenous prostaglandin E_2 can determine the rate of chloride extrusion from the ascending limb of the loop, although this of course does not necessarily imply that endogenous prostaglandins have this effect in-vivo.

Finally the results of the experiments presented in this paper, together with the findings of Danon et al (1978) that hypertonic sodium chloride stimulates PGE production supports the hypothesis proposed in the introduction i.e. that renal prostaglandin synthesis plays a major part in determining the corticomedullary osmotic gradient.

Acknowledgements

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