Br. J. Pharmac. (1984), **82**, 165–173

Effect of bumetanide, frusemide and prostaglandin E_2 on the isolated perfused kidney of rat and rabbit

J.M. Foy & S.Z. Nuhu

Postgraduate School of Studies in Pharmacology, University of Bradford, Bradford, BD7 1DP

1 Rat and rabbit kidneys were isolated, perfused via the renal artery with Krebs solution and perfusion pressure monitored. Dose-response curves to noradrenaline administered as bolus doses or frequency-response curves from transmural arterial electrical stimulation were obtained.

2 A 1 h continuous infusion of bumetanide ($0.1\ \mu g\ ml^{-1}$) increased the sensitivity of rat kidney vessels to noradrenaline, an effect also seen when bumetanide and flurbiprofen ($6\ \mu g\ ml^{-1}$) were simultaneously perfused. In the rabbit there was a decreased sensitivity to both electrical stimulation and noradrenaline.

3 A 1 h continuous infusion of frusemide ($6\ \mu g\ ml^{-1}$) only altered the effects of electrical stimulation. An increased sensitivity in the rat (abolished by flurbiprofen) and a decreased sensitivity in the rabbit kidney was observed.

4 A 1 h continuous infusion of prostaglandin (PG)E_2 ($2\ ng\ ml^{-1}$) increased the sensitivity of rat kidney to both types of stimuli but caused a reduction in the responsiveness of the rabbit kidney to electrical stimuli only. Addition of flurbiprofen only slightly modified these results.

5 The results emphasize and confirm the fundamental difference in reactivity of the rat and rabbit kidney.

6 Bumetanide and frusemide, two ostensibly similar loop diuretics, show significantly different effects on these preparations suggesting that any modification by non-steroidal anti-inflammatory drugs cannot wholly be explained by similar PGE_2 induced haemodynamic changes.

Introduction

Prostaglandins and prostaglandin forming enzymes are widely distributed in the kidney. Initially it was believed that their activity was largely confined to the renal papilla and medulla where all species studied showed a rich source of prostaglandin synthetase (Anggard *et al.*, 1972; Crowshaw & McGiff, 1973; Larsson & Anggard 1973; Blackwell *et al.*, 1975; Dunn 1976). However, it was also shown that the renal cortex could synthesize small amounts of prostaglandin E_2 (PGE_2) and $PGF_{2\alpha}$ (Larsson *et al.*, 1974; Dunn 1976; Pong & Levine 1976). The demonstration of prostaglandin synthetase in cortical and medullary collecting tubules, in arterial vascular endothelial cells and in the interstitial cells of the renal medulla (Smith & Graham, 1978) support suggestions of prostaglandin involvement in the control of water and electrolyte excretion, renal blood flow and possibly blood pressure (Olsen, 1983). Indeed, Malik & McGiff (1975), in their classical study, showed that in both rat and rabbit isolated perfused kidneys, prostaglandins modulate adrenergic transmission. Hedqvist (1981) has put forward evidence suggesting that prostaglandins, particularly PGE_2, are significant trans-synaptic modulators of noradrenergic secretion and may help maintain the functional integrity of the effector organ. Whether the modulation of adrenergic transmission by prostaglandins in the isolated perfused kidney is inhibiting or augmentary appears to depend both on the species (Malik & McGiff, 1975) and on the tone of the renal vasculature (Pace-Asciak & Rosenthal, 1981).

The involvement of loop diuretics with prostaglandins is now well known. Both frusemide (Williamson *et al.*, 1975a,b; Abe *et al.*, 1976; Weber *et al.*, 1977) and bumetanide (Frohlich *et al.*, 1975; Olsen, 1975; Olsen & Ahnfelt-Ronne, 1976; Pedrinelli *et al.*, 1980) have been shown to stimulate renal prostaglandin synthesis and in turn be partially inhibited in their diuretic response by prostaglandin synthetase inhibitors. In this study an attempt is made to compare any possible modulation of adrenergic transmission by frusemide and bumetanide with, in some

cases, the addition of a prostaglandin synthetase inhibitor. Thus isolated perfused kidneys were stimulated either electrically or by addition of noradrenaline to the perfusate in the presence of various combinations of bumetanide, frusemide, PGE_2 and flurbiprofen.

Methods

Male Sprague-Dawley rats weighing 200–400 g and male New Zealand White rabbits weighing 0.8–1.0 kg were used in the experiments. The rats were anaesthetized with pentobarbitone sodium (60 mg kg^{-1}, i.p.) and the rabbits with pentobarbitone sodium (30 mg kg^{-1}, i.v.) via the outer ear vein. The abdomen was exposed by a midline incision and the right kidney, abdominal aorta and renal artery exposed. The abdominal aorta was then ligated above and below the renal artery. A 21 gauge needle was inserted into the renal artery and the artery and kidney flushed with heparinised saline (0.9% w/v NaCl solution) (100 units ml^{-1}). The needle served as both a cannula and an electrode. The kidney was isolated and immediately transferred to a jacketed glass container kept warm by water at 37°C and loosely closed by a perspex lid. The kidney was covered with a tissue moistened with Krebs solution and perfused with Krebs solution of the following composition (mM): NaCl 118.4, KCl 4.09, KH_2PO_4 1.18, $MgSO_4$ 1.18, $CaCl_2$ 2.56, $NaHCO_3$ 25.0 and D-glucose 11.2. The perfusion fluid was maintained at a temperature of 37°C and aerated with 95% O_2 plus 5% CO_2. The rat and rabbit kidneys were perfused at a constant rate of 2 ml min^{-1} and 4 ml min^{-1}, respectively, using a Watson-Marlow pump. The fluid perfusing the kidney flowed from the cut end of the renal vein and the ureter. Changes in perfusion pressure were measured with a Bell and Howell transducer and recorded on a Grass Model 7D Polygraph. The resting kidney perfusion pressure was 50–60 mmHg for the rat and 70–80 mmHg for the rabbit. In all cases there was a stabilization period of 1 h. Following this, dose-response or frequency-response curves were obtained by drug administration or electrical stimulation at 4 min intervals. Frusemide (5.0 μg ml^{-1}), bumetanide (0.1 μg ml^{-1}), PGE_2 (2 ng ml^{-1}) and flurbiprofen (6 μg ml^{-1}) were dissolved in Krebs solution and perfused for 1 h at a rate of 2 ml min^{-1} in the case of the rat kidney and 4 ml min^{-1} in the case of the rabbit kidney. After this a similar test sequence was performed.

An electrode was placed on the renal artery to allow transmural stimulation. Frequency-response curves to electrical stimulation were obtained to increasing frequencies (1–25 Hz) of stimulation (20V, 1 ms pulse width) for periods of 30 s every 4 min. Noradrenaline dose-response curves were obtained in rats (5–100 ng) and rabbits (5–500 ng), each dose being injected into the perfusion system, close to the kidney in a volume of not more than 0.1 ml.

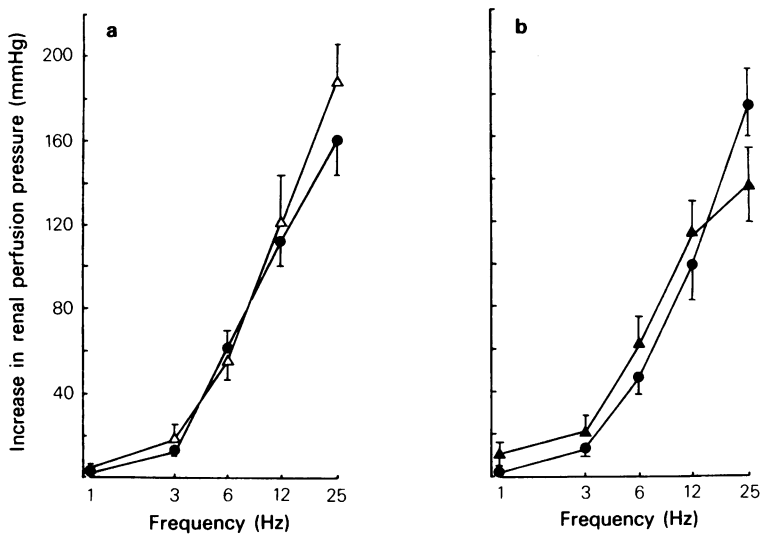

Figure 1 Effect of bumetanide (0.1 μg ml^{-1} infused for one hour) on the increase in renal perfusion pressure in the electrically stimulated isolated perfused rat kidney. (a) Control (●), bumetanide alone (△); (b) control (●), bumetanide + flurbiprofen (6 μg ml^{-1}) (▲). Each point represents the mean ($n = 6$) and the vertical bars show s.e.mean.

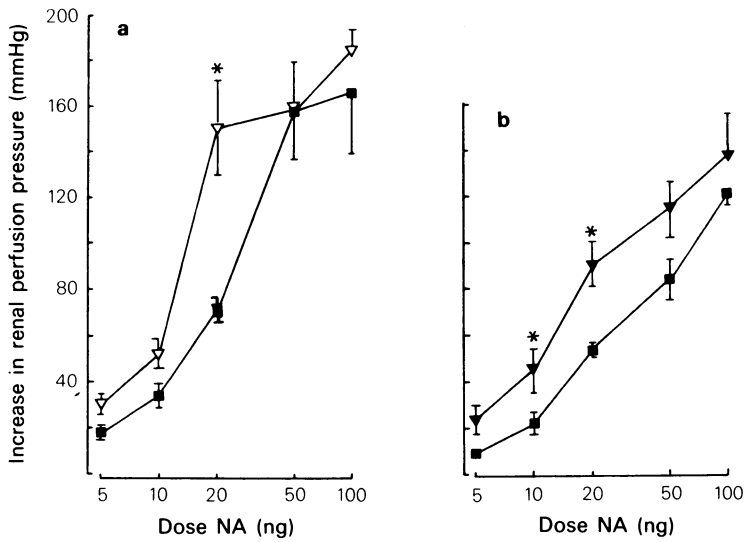

Figure 2 Effect of bumetanide (0.1 $\mu g\,ml^{-1}$ infused for one hour) on the increase in renal perfusion pressure resulting from noradrenaline (NA) injection into the isolated perfused rat kidney. (a) Control (■), bumetanide alone (△); (b) control (■), bumetanide + flurbiprofen (6 $\mu g\,ml^{-1}$) (▼). Each point represents the mean ($n = 6$) and the vertical bars show s.e.mean. * Significantly different from control, $P < 0.05$.

The following drugs were used: noradrenaline bitartrate (Sigma Chemical Co.), prostaglandin (PG) E_2 (Sigma Chemical Co.,), bumetanide (Leo Laboratories Ltd.), frusemide (Hoechst UK Ltd.), sodium flurbiprofen (Boots Co. Ltd.). Except for noradrenaline, they were added to the perfusion fluid to obtain the final concentration. Levels of significance were determined using paired *t* tests.

Results

Electrical stimulation (20 V, 0.1 ms, 1–125 Hz) produced a frequency related increase in resting perfusion pressure in the isolated perfused rat and rabbit kidney. Similar dose-related increases in resting perfusion pressure were also observed to additions of noradrenaline 5–100 ng to the rat kidney and norad-

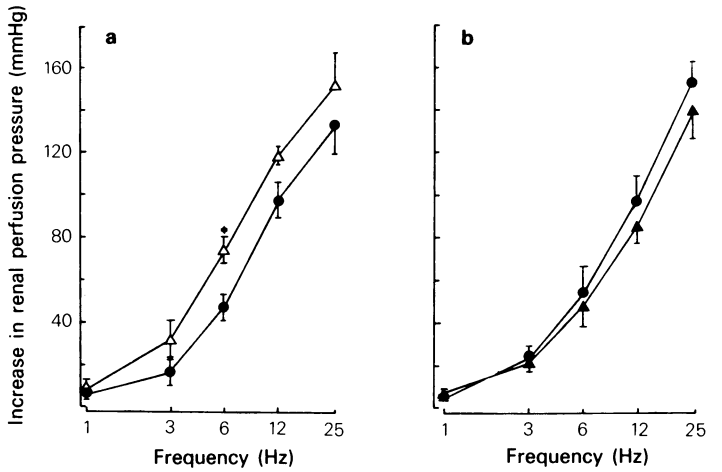

Figure 3 Effect of frusemide (5 $\mu g\,ml^{-1}$ infused for one hour) on the increase in renal perfusion pressure in the electrically stimulated isolated perfused rat kidney. (a) Control (●), frusemide alone (△); (b) control (●) frusemide + flurbiprofen (6 $\mu g\,ml^{-1}$) (▲). Each point represents the mean ($n = 6$) and vertical bars show s.e.mean. * Significantly different from control, $P < 0.05$.

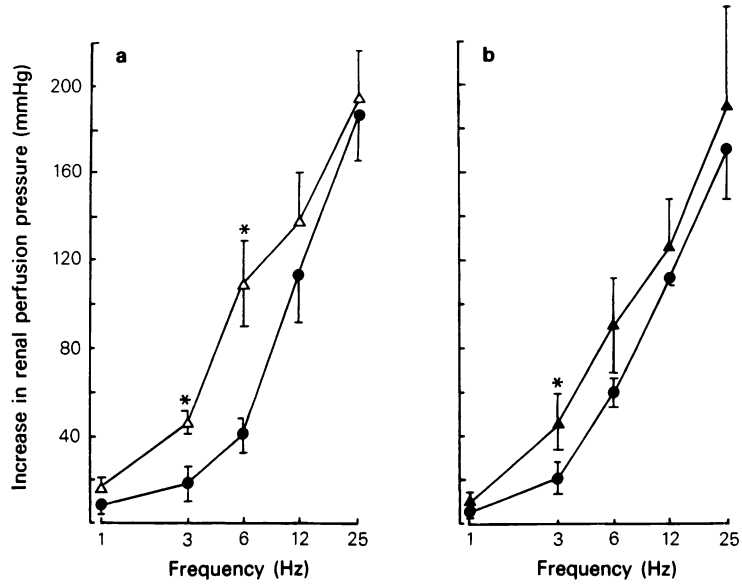

Figure 4 Effect of prostaglandin (PG)E_2 (2 $\mu g\,ml^{-1}$ infused for one hour) on the increase in renal perfusion pressure on the electrically stimulated isolated perfused rat kidney. (a) Control (●), PGE_2 alone (△); (b) control (●), PGE_2 + flurbiprofen (6 $\mu g\,ml^{-1}$) (▲). Each point represents the mean ($n = 6$) and vertical bars show s.e.mean. * Significantly different from control, $P < 0.05$.

renaline 5–500 ng to the rabbit kidney. Control curves did not differ significantly from one another throughout the period of the investigation.

Rat kidney

Continuous infusion of bumetanide (0.1 $\mu g\,ml^{-1}$) for 1 h had no effect on either the resting perfusion pressure or the frequency-response increase in perfusion pressure. Addition of flurbiprofen (6 $\mu g\,ml^{-1}$) to the bumetanide perfused kidneys also failed to affect the curve to electrical stimulation (Figure 1). However, bumetanide did shift the dose-response curve to noradrenaline to the left, both alone and with the addition of flurbiprofen (Figure 2).

A 1 h continuous infusion of frusemide (5.0 $\mu g\,ml^{-1}$) shifted the frequency-response curve to the left, an effect which was not evident when both frusemide and flurbiprofen (6 $\mu g\,ml^{-1}$) were simultaneously perfused (Figure 3). However, in contrast to the effect with bumetanide, neither frusemide alone or in combination with flurbiprofen affected the dose-response curves to noradrenaline. A 1 h continuous infusion of PGE_2 (2 $ng\,ml^{-1}$) shifted the frequency-response curve to the left as did an infusion of PGE_2 plus flurbiprofen (6 $\mu g\,ml^{-1}$), though to a less marked extent (Figure 4). Similar leftward shifts of the dose-response curves to noradrenaline were seen, both with and without the addition of flurbiprofen.

Continuous perfusion of flurbiprofen (6 $\mu g\,ml^{-1}$) alone for 1 h showed no effect on electrical stimulation but shifted the dose-response curve to noradrenaline to the left, the sensitivity of the preparation being increased to all but the highest dose (Figure 5).

Rabbit kidney

Infusion of bumetanide (0.1 $\mu g\,ml^{-1}$) for 1 h in the rabbit kidney shifted the frequency-response curve to the right, thus producing a significantly decreased responsiveness. When flurbiprofen was added to the bumetanide infusion the decreased responsiveness appeared attenuated, being significantly different from control at 6 Hz only (Figure 6). Similarly, infusion of bumetanide and bumetanide plus flurbiprofen (6 $\mu g\,ml^{-1}$) both reduced responsiveness of the preparation to noradrenaline, producing significant rightward shifts of the dose-response curves (Figure 7).

A 1 h continuous infusion of frusemide (5 $\mu g\,ml^{-1}$) and frusemide plus flurbiprofen (6 $\mu g\,ml^{-1}$) both produced significant shifts to the right of the frequency-response curves (Figure 8). In contrast, similar treatments had no effect on the dose-response curves to noradrenaline.

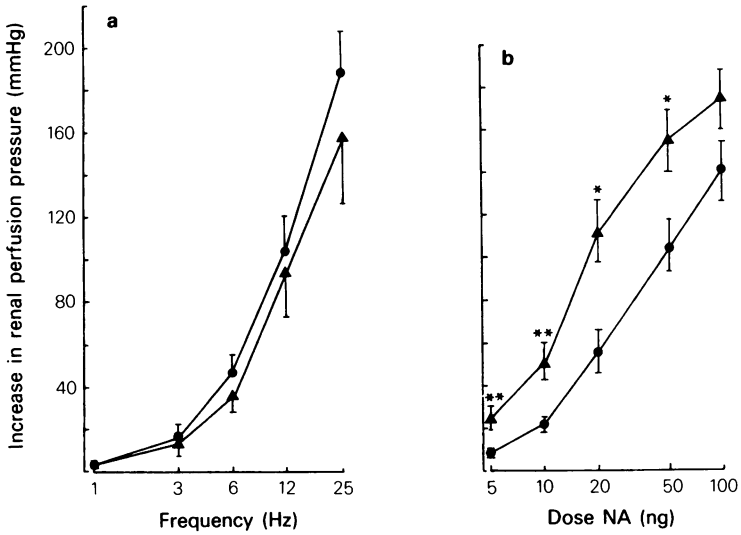

Figure 5 Effect of flurbiprofen (6 $\mu g\,ml^{-1}$ infused for one hour) on the increase in renal perfusion pressure in the (a) electrically and (b) noradrenaline stimulated isolated perfused rat kidney. Control (●), flurbiprofen (▲). Each point represents the mean ($n = 6$) and vertical bars show s.e.mean. * $P < 0.05$; ** $P < 0.01$, significantly different from control.

A 1 h continuous infusion of PGE_2 (2 $ng\,ml^{-1}$) and PGE_2 plus flurbiprofen (6 $\mu g\,ml^{-1}$) both significantly decreased the electrical responses at 6 Hz (Figure 9) but neither treatment affected the dose-response relationship to noradrenaline.

A 1 h continuous infusion of flurbiprofen alone

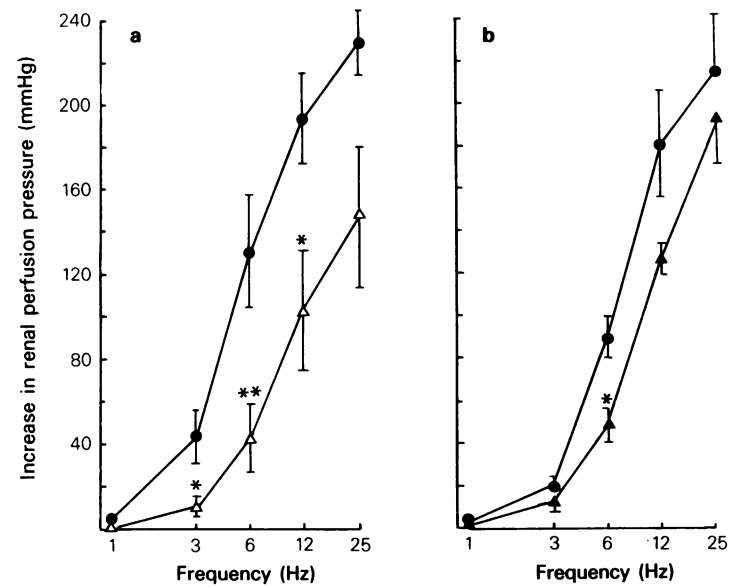

Figure 6 Effect of bumetanide (0.1 $\mu g\,ml^{-1}$ infused for one hour) on the increase in renal perfusion pressure in the electrically stimulated isolated perfused rabbit kidney. (a) Control (●), bumetanide alone (△); (b) control (●), bumetanide + flurbiprofen (6 $\mu g\,ml^{-1}$) (▲). Each point represents the mean ($n = 6$) and vertical bars show s.e.mean. * $P < 0.05$; ** $P < 0.01$, significantly different from control.

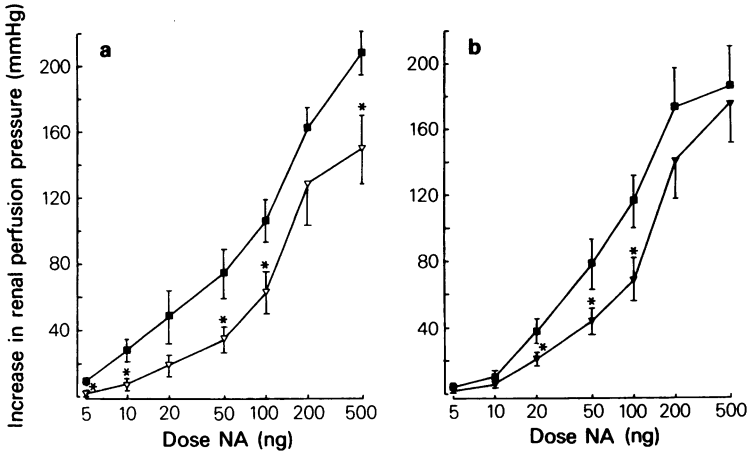

Figure 7 Effect of bumetanide (0.1 $\mu g\,ml^{-1}$ infused for one hour) on the increase in renal perfusion pressure resulting from a noradrenaline (NA) injection into the isolated perfused rabbit kidney. (a) Control (■), bumetanide alone (△); (b) control (■), bumetanide + flurbiprofen (6 $\mu g\,ml^{-1}$) (▼). Each point represents the mean ($n = 6$) and vertical bars show s.e.mean. * Significantly different from control, $P < 0.05$.

produced an increase in the response to 3 Hz and 20 ng noradrenaline, only.

None of the treatments employed, i.e. diuretics, PGE_2 or flurbiprofen, had any effect on the resting perfusion pressure of the isolated rat or rabbit kidney.

Discussion

In this study, within the limits of the parameters employed, a direct comparison is possible between rat and rabbit isolated perfused kidney and any possible haemodynamic (as opposed to diuretic) action of

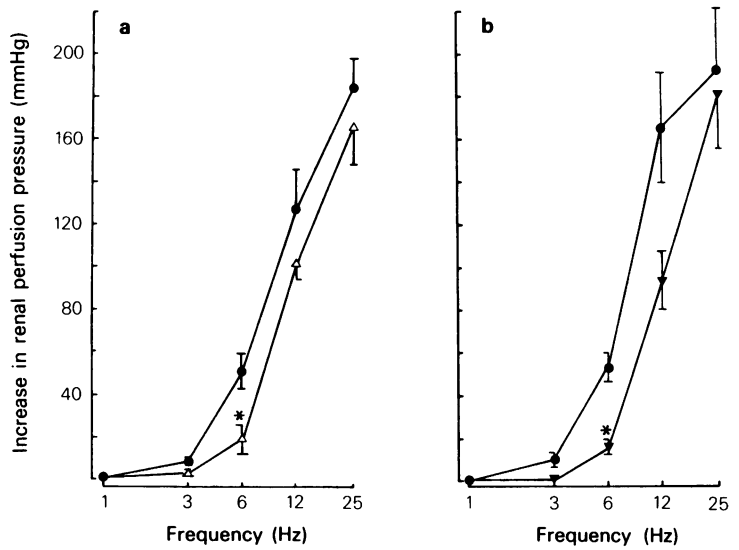

Figure 8 Effect of frusemide (5 $\mu g\,ml^{-1}$ infused for one hour) on the increase in renal perfusion pressure in the electrically stimulated isolated perfused rabbit kidney. (a) Control (●), frusemide alone (△); (b) control (●), frusemide + flurbiprofen (6 $\mu g\,ml^{-1}$) (▼). Each point represents the mean ($n = 6$) and vertical bars show s.e.mean. * Significantly different from control, $P < 0.05$.

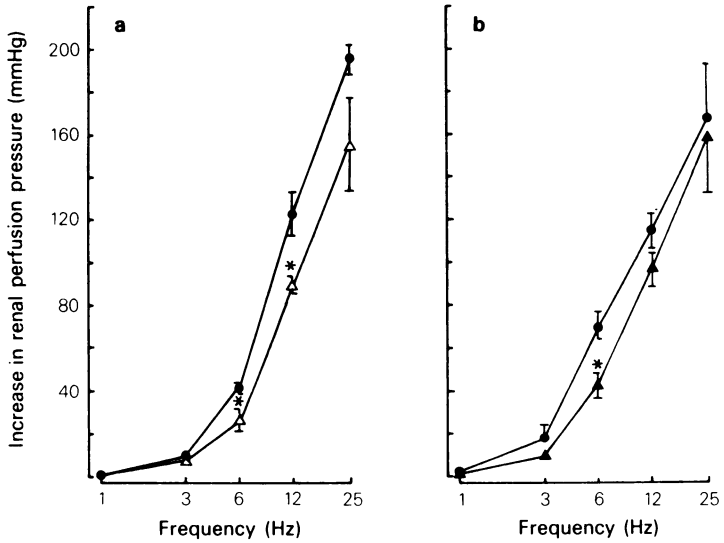

Figure 9 Effect of prostaglandin (PG)E_2 ($2\,\mu g\,ml^{-1}$ infused for one hour) on the increase in renal perfusion pressure in the electrically stimulated isolated perfused rabbit kidney. (a) Control (●), PGE_2 alone (△); (b) control (●), PGE_2 + flurbiprofen ($6\,\mu g\,ml^{-1}$) (▲). Each point represents the mean ($n = 6$) and vertical bars show s.e.mean. * Significantly different from control, $P < 0.05$.

two ostensibly similar loop diuretics. The concentrations of diuretics, PGE_2 and flurbiprofen chosen were those either shown, in previous studies, to be effective as diuretics or used in previous kidney perfusion studies (Malik & McGiff, 1975; Ferrando *et al.*, 1981). There can now be little doubt that prostaglandins play an important role in the kidney, the significance of which probably varies between species. In most instances PGE_2 and PGI_2 are thought to enhance renal blood flow, glomerular filtration rate and renin secretion and inhibit Na^+ and Cl^- reabsorption and (for PGE_2 only) the action of ADH (Smith, 1983). In general, and by contrast, thromboxanes have actions opposite to the above. In this study only the effect of PGE_2 was investigated, together with an irreversible prostaglandin biosynthesis inhibitor used at a concentration known to inhibit synthesis without necessarily acting as an antagonist (Froben, 1981).

On comparing the effect of electrical stimulation and noradrenaline administration overall in the rat and rabbit kidney, it can be clearly seen that in the rat there was an enhancement of arterial tone, whereas in the rabbit the opposite occurred. For example, in the rat PGE_2 infusion shifted both electrical and noradrenaline curves to the left whereas in the rabbit the shift was to the right, in the electrically stimulated preparation only. This result confirms the essential difference between the two species first demonstrated by Malik & McGiff (1975). In rabbit and perhaps human kidney prostaglandins of the E series had been proposed as physiological 'braking mechanisms' to the release of adrenergic transmitters (Hedqvist, 1970). This hypothesis would certainly fit the current results where flurbiprofen, having blocked endogenous synthesis, enhanced both electrical and transmitter responses in the rabbit organ although not to a very marked extent. In the presence of PGE_2 and either of the two diuretics responsiveness was suppressed. In the rat there was a less consistent response to the various procedures but it always tended towards vasoconstriction. Both PGE_2 and flubiprofen infusion enhanced the constrictor effects of noradrenaline, thus suggesting a more important role in transmitter modulation for other vasoactive substances in the rat kidney. However, very recently Pace-Asciak & Rosenthal (1983) provided another possible explanation for the apparently unusual effects of PGE_2 in the rat. They showed that when the renal vessel resistance is raised, using vasopressin or angiotensin II and the flow rate increased (but still within physiological limits), PGE_2 reveals vasodilator properties which are presumably masked in the relatively relaxed vessels of this study perfused at only one third of the volume employed by Pace-Asciak & Rosenthal. Perhaps the sensitivity of the rat kidney to changes in experimental condition renders it a less than ideal preparation for studies involving prostaglandins.

Turning to the effects of the loop diuretics, the question here is not whether the action of these drugs is modified by cyclo-oxygenase inhibitors or indeed

whether interactions occur at a haemodynamic level – both are well documented (Olsen, 1983), but whether there are important differences between the two. *In vitro* frusemide has been shown to inhibit 15-hydroxyprostaglandin dehydoxenase (Stone & Hart, 1976) while bumetanide had negligible activity (Oliw, 1979). It has also been proposed that frusemide may act sooner in the synthesis of PGs at the stage of de-esterification of arachidonic acid. This biochemical view is challenged by the physiological explanation which suggests that diuretics produce an intrarenal hydrodynamic state somewhat akin to ureteral obstruction. The increase in proximal tubular pressure reduces glomerular filtration which leads to the activation of cortical prostaglandins. Either of the above hypotheses could be applied to the present study, where of the eight sets of experiments (in both species) in which bumetanide was employed, shifts in the response curve were seen on six occasions but only on three occasions with frusemide. Studies using bumetanide in the rat have been complicated by the apparently unique ability of this species to deactivate the drug by metabolism (Ings & Stevens, 1982). However, in this study metabolic deactivation should not be a problem using a constant infusion through an isolated organ. If the effects of the diuretics could be attributed entirely to the haemodynamic consequences of PGE_2 release then they should have similar effects to PGE_2 infusion. This is only partly the case. For instance, in the rat PGE_2 enhances the responses to both electrical stimulation and noradrenaline administration, whereas bumetanide alters only the latter. In contrast, frusemide does potentiate the responses to electrical stimulation (an effect which is abolished by simultaneous flurbiprofen administration) but not to noradrenaline. In the rabbit bumetanide attenuates the responses to both electrical stimulation and transmitter and this effect is little affected by flurbiprofen. Frusemide only attenuates responses to electrical stimulation. It is concluded therefore that there are significant differences between the two diuretics and that their renal effects as modified by non-steroidal anti-inflammatory drugs cannot wholly be explained on the basis of similar PGE_2 induced haemodynamic changes. What interactions they may have with other prostaglandins on this organ remains an open question.

References

ABE, K., OTSUKA, Y., YASUJIMA, M., CIBA, S., SEINO, M., IROKANA, N. & YOSHINAGI, K. (1976). Metabolism of PG in man: Effect of frusemide on the excretion of the main metabolite of PGF_{2x}. *Prostaglandins*, **12**, 843–848.

ANGGARD, E., BOHMAN, S.O., GRIFFIN, J.E., LARSSON, C. & MAUNSBAUCH, A.B. (1972). Subcellular localisation of the prostaglandin system in the rabbit renal papilla. *Acta physiol. scand.*, **84**, 231–246.

BLACKWELL, G.J., FLOWER, R.J. & VANE, J.R. (1975). Some characteristics of the prostaglandin synthesizing system in rabbit kidney microsomes. *Biochim. biophys. Acta*, **398**, 178–190.

CROWSHAW, K. & McGIFF, J.C. (1973). Prostaglandins in the kidney: a correlative study of their biochemistry and renal function. In *Mechanisms of Hypertension*, ed. Sambhi, M.P. pp. 254–273, New York: Elsevier.

DUNN, M.J. (1976). Renal prostaglandin synthesis in the spontaneously hypertensive rat. *J. clin. Invest.*, **58**, 862–870.

FERRANDO, C., FOY, J.M., PRATT, C.N.F.W. & PURVIS, J.R. (1981). On the pharmacological actions of a diuretic, fenquizone, with particular reference to its site of action. *J. Pharm. Pharmac.*, **33**, 219–222.

FROBEN (1981). Clinical and technical review. Nottingham: The Boots Company Ltd.

FROLICH, J.C., WILSON, T.W., SWEETMAN, B.J., SONIGEL, M., NIES, A.S., CARR, K., WATSON, J.T. & OATES, J.A. (1975). Urinary prostaglandins: identification and origin. *J. clin. Invest.*, **55**, 763–770.

HEDQVIST, P. (1970). Studies on the effect of prostaglandins E_1 and E_2 on the sympathetic neuromuscular transmission in some animal tissues. *Acta physiol. scand.*, **79**, 1–40.

HEDQVIST, P. (1981). Trans-synaptic modulation versus α-autoinhibition of noradrenaline secretion. In *Chemical Neurotransmission: 75 years*, ed. Stjärne, L.H., Hedqvist, P. & Wennmalm, A., pp. 223–233, London: Academic Press.

INGS, R.M.J. & STEVENS, L.A. (1982). Pharmacokinetics and metabolism of diuretics. *Prog. Drug Metab.*, **7**, 57–171.

LARSSON, C. & ANGGARD, E. (1973). Regional differences in the formation and metabolism of prostaglandins in the rabbit kidney. *Eur. J. Pharmac.*, **21**, 30–36.

LARSSON, C., WEBER, P. & ANGGARD, E. (1974). Arachidonic acid increases and indomethacin decreases plasma renin activity in the rabbit. *Eur. J. Pharmac.*, **28**, 391–394.

MALIK, K.U. & McGIFF, J.C. (1975). Modulation by prostaglandins of adrenergic transmission in the isolated perfused rabbit and rat kidney. *Circulation Res.*, **36**, 599–609.

OLIW, E. (1979). Prostaglandins and kidney function. An experimental study in the rabbit. *Acta physiol. scand. [Suppl.]*, **461**, 1–55.

OLSEN, U.B. & AHNFELT-RONNE, T. (1976). Bumetanide induced increase of renal blood flow in conscious dogs and its relations to local renal hormones (PGE, Kallikrein and Renin). *Acta pharmac. tox.*, **38**, 219–228.

OLSEN, U.B. (1975). Indomethacin inhibition of bumetanide diuresis in dogs. *Acta pharmac. tox.*, **37**, 65–78.

OLSEN, U.B. (1983). Diuretics and kidney prostaglandins. In *Prostaglandins and the kidney*, ed. Dunn, M.J., Patrono, C. & Cinotti, C.A., pp. 205–212, London: Plenum.

PACE-ASCIAK, C.R. & ROSENTHAL, A. (1981). Opposition

of the vasopressin-induced vasoconstriction in the isolated perfused rat kidney by some prostaglandins. *Prostaglandins*, **22,** 567–574.

PACE-ASCIAK, C.R. & ROSENTHAL, A. (1983). Reversal of vasoconstriction in the isolated perfused rat kidney by picogram amounts of PGE_2. In *Prostaglandins and the kidney*, ed. Dunn, M.J., Patrono, C. & Cinotti, C.A., pp. 119–123, London: Plenum.

PEDRINELLI, R., MAGAGNA, A., ARZILLI, F., SASSANO, P. & SALVETTI, A. (1980). Influence of indomethacin on the natriuretic and renin-stimulating effect of bumetanide in essential hypertension. *Clin. Pharmac. Ther.*, **28,** 722–731.

PONG, S.S. & LEVINE, L. (1976). Biosynthesis of prostaglandins in rabbit renal cortex. *Res. Comm. Chem. Pathol. Pharmac.*, **13,** 115–123.

SMITH, W.L. (1983). Endogenous agents affecting kidney function: their interrelationships modulation and control. In *Diuretics*, ed. Cragoe, E.J. pp. 575–651. New York: Wiley.

SMITH, W.L. & GRAHAM, T.B. (1978). Immunohistochemical localisation of the prostaglandin-forming cyclooxygenase in renal cortex. *Am. J. Physiol.*, **235,** F451–457.

STONE, K.J. & HART, M. (1976). Inhibition of renal PGE_2-9-ketoreductase by diuretics. *Prostaglandins*, **12,** 197–207.

WEBER, P.C., SCHERER, B. & LARSSON, C. (1977). Increase of free arachidonic acid by frusemide in man as the cause of prostaglandin and renin release. *Eur. J. Pharmac.*, **41,** 329–332.

WILLIAMSON, H.E., BOURLAND, W.A. & MARCHAND, G.R. (1975a). Inhibition of frusemide-induced increase in renal blood flow by indomethacin. *Proc. Soc. exp. Biol. Med.*, **148,** 164–165.

WILLIAMSON, H.E., BOURLAND, W.A., MARCHAND, G.R. & VAN ORDEN, D.E. (1975b). Frusemide induced release of prostaglandins E to increase renal blood flow. *Proc. Soc. exp. Biol. Med.*, **150,** 104–106.

(*Received September 20, 1983.*
Revised December 2, 1983.)