Br. J. Pharmac. (1977), **59**, 571–575

RENAL BLOOD FLOW AUTOREGULATION AND RENAL VENOUS PROSTAGLANDINS IN THE PUMP-PERFUSED CANINE KIDNEY (*In situ*)

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1 Renal autoregulation of blood flow was re-examined in the pump-perfused canine kidney and concentrations of prostaglandins E and F in the renal venous plasma were measured by radioimmunoassay.

2 At low perfusion pressures, below the range of autoregulation, prostaglandin E and F concentrations rose and calculated prostaglandin E secretion rate fell.

3 Meclofenamate (10 mg/kg, i.v.) reduced renal blood flow and prostaglandin E and F secretion rates, but did not abolish autoregulation.

4 Renal prostaglandins do not appear to mediate autoregulation in the kidney but may affect the level at which flow is controlled.

Introduction

The mechanism of renal blood flow autoregulation has escaped elucidation despite many attempts to implicate such factors as plasma skimming (Kinter & Pappenheimer, 1956), myogenic responses (Waugh, 1958) and tissue pressure (Hinshaw, Ballus, Day & Carlson, 1959). A capillaron model has also been proposed (Murao & Rodbard, 1976) but fails to predict why there is an upper limit to autoregulation. Herbaczynska-Cedro & Vane (1973), using pump-perfused kidneys, demonstrated the abolition of autoregulation and output of prostaglandin-like substances by indomethacin, implying prostaglandin E as a mediator of autoregulation. This was subsequently challenged. Owen, Ehrhart, Weidner, Haddy & Scott (1974) failed to abolish autoregulation in the naturally perfused kidney using indomethacin. Venuto, O'Dorisio, Ferris & Stein (1975) and Anderson, Taher, Cronin, McDonald & Schrier (1975) also failed to abolish autoregulation using meclofenamate as well as indomethacin.

This discrepancy has been assigned to a methodological difference, namely the large resting resistance to flow seen in the pump-perfused kidney (Venuto *et al.*, 1975; Anderson *et al.*, 1975). We re-examined the role of prostaglandins of the E and F series in the pump-perfused canine kidney using meclofenamate to inhibit synthesis, and radioimmunoassay to estimate renal venous concentrations of both prostaglandins.

Methods

Ten mongrel dogs of either sex, weighing between 8 and 15.5 kg were used. They were anaesthetized with pentobarbitone (30 mg/kg, i.v.) and surgical diathermy was used in subsequent operative procedures (there was no significant blood loss). Each animal was tracheotomized and systemic blood pressure was measured from the left femoral artery. Temperature was held at 37°C by a heating pad controlled by a thermistor rectal probe. A midline laparotomy was performed and a catheter placed in the left renal vein via a femoral vein, for renal venous blood sampling. The left ovarian or spermatic vein was ligated. Hypotonic saline (0.8% w/v NaCl solution) was infused intravenously at about 2 ml/min to ensure adequate urine flow. The animal was injected with heparin (1000 u/kg) and blood removed from the right carotid artery by a slowly revolving Watson Marlow MHRE pump. Part of this flow passed through a cannulating flow probe (Statham, 2.00 mm lumen) and was carried by silicone tubing through an incision in the abdominal wall to the catheterized left renal artery. Renal artery pressure (RAP) was measured at the tip of this catheter through a fine tube.

The remaining flow was shunted through a Starling resistance to a femoral vein. Using compressed air, with a controllable leak, the pressure in the Starling resistance box and so the mean perfusion pressure of the kidney could be controlled, while the pulse pressure remained about 25 mmHg. With this system

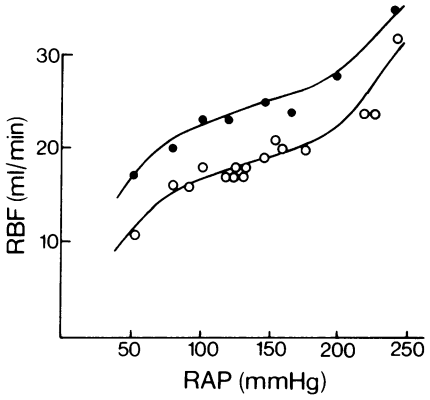

Figure 1 This figure shows the individual results of one experiment (expt. 7) before (●) and after (○) meclofenamate. RBF = renal blood flow; RAP = renal artery pressure. The lines through the points were obtained from the cubic expression of each set of points; $y = 24.9 + 0.040(x-141) + 5.26(x-141)^3$ for control and $y = 18.5 + 0.037(x-137) + 6.46(x-137)^3$ for the meclofenamate curve.

the kidney could be perfused at a range of pressures while the pump ran at a constant slow speed, thus preventing damage to the blood at high pump speeds.

All pressures were measured with Consolidated Electrodynamic L223 transducers and the electronically averaged pressures and renal blood flow signals were recorded on light-sensitive paper (Honeywell, Visicorder 2206). Pressure flow curves were obtained by varying RAP every 5 min, since autoregulation was complete within 3 minutes. After a control curve was obtained, meclofenamate (10 mg/kg, i.v.) dissolved in 10 ml of 0.9% saline was injected and after 15 to 20 min another pressure flow curve was recorded.

Samples of renal venous blood (10 ml) were removed at 50, 100, 150 and 200 mmHg during the determination of the control pressure flow curve and at 100 mmHg after meclofenamate in 4 experiments. The samples were collected in ice cold centrifuge tubes and spun at 4°C for 25 min at 15,000 *g*. The haematocrit of each sample was noted for estimation of renal plasma flow and so prostaglandin secretion rate. The plasma was stored in a freezer for less than 3 weeks before the assay. The plasma was acidified and the prostaglandins extracted and purified by silicic acid column chromatography before radioimmunoassay.

Prostaglandin antibody was raised in rabbits with prostaglandin E_2 conjugated to thyroglobulin by means of the carbodiimide reaction. The antibody exhibited a cross-reaction of 100% with prostaglandin E_1, 2% with prostaglandin A and B groups and 5% with 13, 14 dihydro-15-keto prostaglandin E_2 and was used at a final concentration of 1/2,000. The sensitivity was 30 pg/tube (60 pg/ml of sample), with an intra-assay precision of about 15%.

Prostaglandin F was measured by means of the antibody raised in rabbits to prostaglandin $F_{2\alpha}$ bovine serum albumin conjugate (Dighe, Emslie, Henderson, Rutherford & Simon, 1975). The antibody showed a cross-reaction of 100% with prostaglandin $F_{1\alpha}$, 2.6% with $F_{2\beta}$ and 2% with D_2. The other prostaglandin groups and metabolites cross-reacted less than 1%. The antibody was used at a final dilution of 1/35,000 and the sensitivity was 45 pg/tube (90 pg/ml of sample), with an intra-assay precision of about 15%. Recovery of both prostaglandins was about 60% and the reported levels were corrected for recovery.

The pressure flow curves were plotted for each individual experiment after fitting the data by computer to a cubic expression, which describes the general shape of the autoregulation curve:

$$y = p + q(x-m) + r(x-m)^3$$

where p is the flow during autoregulation (point of inflexion), m is the pressure at this point, q is the slope of the plateau part of the autoregulation curve, r is a constant, x is the pressure at any point and y is the flow at any point.

Figure 1 demonstrates the results of one experiment (expt. 7) to which the cubic expression has been fitted by the computer using the method of least squares.

A straight regression line was also fitted to the experimental points. In the absence of autoregulation, the plateau gradient (q) would approximate to the regression coefficient (β) such that q/β would tend to unity. Values of q/β less than one or even negative were taken as objective evidence for autoregulation.

Values are expressed as means ± s.e. and the results were analysed by Student's *t* test; probabilities less than 5% were accepted as being statistically significant.

Results

Mean systolic pressure (101 ± 5 mmHg) was well maintained throughout each experiment. Two experiments showed no autoregulation and a third lost autoregulation before completion of the control period. In the remaining 7 experiments autoregulation was obtained. Mean autoregulation flow was 54 ± 13 ml/min ($n=7$) and meclofenamate (10 mg/kg, i.v.) reduced it to 26 ± 6 ml/min ($n=6$, $P<0.05$). Autoregulation was lost in one experiment after meclofenamate. The mean autoregulation flow corrected for kidney weight was 1.6 ± 0.4 ml/min per g kidney, which is about 30% lower than that reported by Ono, Kokubun & Hashimoto (1974).

Midpoint autoregulation pressure (m) did not change after meclofenamate, 133 ± 5 mmHg before and 144 ± 10 mmHg after. The ratio q/β was always

less than unity before and after meclofenamate (except in one experiment where autoregulation was lost after meclofenamate), indicating preservation of autoregulation. See Table 1 for the haemodynamic data.

There was a tendency for renal venous prostaglandin E concentrations to rise and calculated secretion rates (the product of the concentrations and their respective renal plasma flows) to fall as RAP was reduced. Using absolute values no significance could be demonstrated due to the large variation in values between experiments (Table 2). By expressing the values at 50, 100 and 150 mmHg as ratios of those at 200 mmHg, a statistically significant rise in concentration of prostaglandin E was seen at the lowest pressure of 50 mmHg ($P<0.05$) and the calculated secretion rate fell ($P<0.01$).

Prostaglandin F concentrations expressed in a similar manner also rose as pressure fell, reaching statistical significance at the lowest pressure ($P<0.05$). However, prostaglandin F secretion did not change.

Meclofenamate (10 mg/kg) produced a significant

Table 1 Renal haemodynamics before and after meclofenamate

Experiment	*m*	*q*	*β*	*p*	*q/β*
1A	116	0.012	0.076	29.2	0.158
1B	132	0.057	0.095	13.4	0.600
2A	133	−0.027	0.036	25.4	−0.750
2B	184	0.025	0.033	10.7	0.757
3A	120	0.016	0.180	33.9	0.089
3B	155	0.147	0.175	25.9	0.840
4A	150	0.070	0.360	73.0	0.194
4B	113	0.111	0.229	41.5	0.484
5A	145	0.059	0.538	112.7	0.110
5B	144	0.048	0.130	46.3	0.369
6A	127	0.060	0.261	80.8	0.229
6B	(42)	(0.24)	0.17	(6.97)	(1.43)
7A	141	0.040	0.080	24.9	0.560
7B	137	0.037	0.083	18.5	0.446

A–control values; B–values after meclofenamate. m = autoregulation midpoint pressure (mmHg); q = slope of autoregulation point of curve; β = linear regression coefficient; p is the autoregulation flow (ml/min); q/β is an index of autoregulation. In the absence of autoregulation this value tends to unity.
In expt. 6 autoregulation was lost after meclofenamate and the pressure-flow curve becoming virtually rectilinear. The derived parameters except β have little meaning.

Table 2 Prostaglandin E (PGE) and prostaglandin F (PGF) renal venous plasma concentrations and calculated secretion rates at different renal artery pressures (RAP).

		PGE concentration		*PGE secretion rate*	
RAP (mmHg)	n	*pg/ml*	*ratios*	*ng/min*	*ratios*
50	6	542 ± 222	1.40 ± 0.17*	11.31 ± 4.83	0.65 ± 0.12†
100	5	311 ± 47	1.11 ± 0.25	10.77 ± 2.91	0.80 ± 0.21
150	5	398 ± 127	1.04 ± 0.17	19.47 ± 7.71	0.97 ± 0.25
200	6	360 ± 110	1	15.99 ± 4.63	1
		PGF concentration		*PGF secretion rate*	
		pg/ml	*ratios*	*ng/min*	*ratios*
50	4	800 ± 353	2.24 ± 0.49*	17.53 ± 7.24	1.04 ± 0.18
100	4	237 ± 67	1.07 ± 0.33	10.79 ± 3.84	0.92 ± 0.30
150	4	363 ± 105	1.33 ± 0.47	25.20 ± 9.07	1.82 ± 0.72
200	4	368 ± 173	1	16.37 ± 5.62	1

Absolute values are given and ratios relative to those values at 200 mmHg. *n* is the number of observations.
* $P<0.05$ † $P<0.01$

fall in the calculated secretion rate of prostaglandin E, at a perfusion pressure of 150 mmHg, from 15.65 ± 4.43 ng/min ($n=8$) to 3.61 ± 0.61 ng/min ($n=8$, $P<0.05$). Renal venous plasma concentration also fell, from 315 ± 58 pg/ml to 178 ± 26 pg/ml ($P<0.05$). Prostaglandin F showed a similar response, with a fall in the secretion rate from 18.93 ± 5.44 ng/min ($n=7$) to 2.73 ± 0.27 ng/min ($n=7$, $P<0.01$) and in concentration from 314 ± 57 pg/ml to 134 ± 23 pg/ml ($P<0.01$).

Discussion

Renal blood flow in the anaesthetized dog is dependent on prostaglandin synthesis, particularly of prostaglandin E_2 (Lonigro, Itskovitz, Crowshaw & McGiff, 1973). Infusion of the prostaglandin E and A series into the canine renal artery increases renal blood flow (Fülgraff, Brandenbusch & Heintze, 1974; Tannenbaum, Splawinskii, Oates & Nies, 1975). Arachidonic acid also elevates flow and prostaglandin synthesis inhibition prevents this (Tannenbaum *et al.*, 1975).

Renal prostaglandins also appear to be involved in intrarenal blood flow distribution in the dog (Itskovitz, Stemper, Pacholczyk & McGiff, 1973; Chang, Splawinskii, Oates & Nies, 1975) and in the rabbit (Larsson & Ånggärd, 1974).

Acute renal ischaemia increases prostaglandin E-like material present in the renal vein (McGiff, Crowshaw, Terragno, Lonigro, Strand, Williamson, Lee & Ng, 1970). Herbaczynska-Cedro & Vane (1973) confirmed this and showed that indomethacin prevented this rise in concentration and also prevented autoregulation. This latter study implied that prostaglandin E was released in response to a lowered RAP and that this mediated autoregulation.

Beckman & Zehr (1975), using radioimmunoassay, showed that renal venous prostaglandin E concentration rose when renal blood flow was reduced, but this was due to a dilution effect. A 50% reduction in flow resulted in a rise in concentration but less than double the control concentration, which must be interpreted as a fall in secretion rate.

In calculating secretion rate, it is assumed that the arterial prostaglandins presented to the kidney are removed in passage (Aiken & Vane, 1973) so that all the renal venous prostaglandins can be assumed to have been newly synthesized by the kidney. It is possible that the intrarenal metabolism of prostaglandins is flow-dependent, decreasing in efficiency as the flow increases.

In the naturally perfused kidney, prostaglandin synthetase inhibitors do not abolish renal blood flow autoregulation (Owen *et al.*, 1974; Venuto *et al.*, 1975; Anderson *et al.*, 1975). The latter two groups suggest that the large resting resistance seen in the pump-perfused kidney (Herbaczynska-Cedro & Vane, 1973) may lead to prostaglandin-dependent autoregulation. This cannot be the case since despite inhibition of prostaglandin synthesis, 6 out of 7 of our experiments still showed autoregulation. Prostaglandin E secretion was reduced by about 70%, in agreement with Venuto *et al.* (1975). It is possible that although secretion rate was greatly reduced, the smaller reduction in concentration (due to the concomitant fall in flow) was not enough to abolish autoregulation. It is possible that intrarenal blood prostaglandin concentration rather than secretion rate *per se* is important in determining intrarenal haemodynamics.

In the conscious dog, indomethacin (2 mg/kg) did not affect blood pressure, renal blood flow or intrarenal blood flow distribution (Zins, 1975) and meclofenamate and the competitive synthetase inhibitor Ro 20–5720 (1 mg/kg) did not alter blood pressure or renal blood flow (Kirschenbaum & Stein, 1976). However, in these studies, inhibition was not examined.

Renal blood flow and intrarenal haemodynamics may be dependent on prostaglandin synthesis in the anaesthetized dog due to the elevated prostaglandin E concentrations. In 7 conscious dogs we have obtained resting renal venous plasma concentrations of prostaglandin E of 191 ± 25 pg/ml ($n=10$), levels significantly lower than those reported in the anaesthetized dogs at a pressure of 100 mmHg, 361 ± 63 pg/ml ($n=10$, $P<0.05$). Levels may be higher in the anaesthetized animal for several reasons. The high resting resistance in the pump-perfused kidney will lower flow and so raise concentrations through a diluting mechanism. Angiotensin is released during barbiturate anaesthesia and this lowers renal blood flow (Burger, Hopkins, Tulloch & Hollenburg, 1976) and will also cause a direct release of prostaglandins (McGiff, Crowshaw, Terragno & Lonigro, 1970).

The mere introduction of the superfusion bioassay system as used by Herbaczynska-Cedro & Vane (1973) has been reported to elevate arterial and renal venous prostaglandin E, possibly due to kinin formation (Satch & Zimmerman, 1976). It is possible that foreign surfaces in general, such as those of the pump circuit tubing, may have a similar effect.

In conclusion, renal prostaglandins do not appear to mediate autoregulation of renal blood flow in the pump-perfused kidney, but may play a role in determining absolute blood flow, at least in the anaesthetized dog.

We would like to thank Mr P.H. Whelpdale for his excellent technical assistance and Dr R. Elton for his statistical advice and help. G.W.S. is an MRC scholar.

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(Received July 26, 1976. Revised November 8, 1976.)