The Journal of Experimental Biology 202, 947–955 (1999)
Printed in Great Britain © The Company of Biologists Limited 1999
JEB1901

SECRETION OF PRIMARY URINE BY GLOMERULI OF THE HAGFISH KIDNEY

J. A. RIEGEL

Department of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3EJ, UK

e-mail: jar1004@hermes.cam.ac.uk

Accepted 5 January; published on WWW 22 March 1999

Summary

Adrenaline and noradrenaline increased the perfusion pressure (P_{perf}) and single glomerulus filtration rate (SGFR) of perfused hagfish glomeruli. Small amounts (0.1 % or 0.5 %) of bovine serum albumin (BSA) in perfusion fluids containing Ficoll 70 did not diminish the loss of colloid from hagfish glomerular capillaries as has been reported for other perfused capillaries. However, replacement of Ficoll 70 with an osmotically equivalent amount (3 %) of BSA appreciably reduced colloid loss. It was concluded that adrenaline and colloids enhanced flow through the urine-forming capillaries. Whereas adrenaline elevated the SGFR, colloid lowered the SGFR probably by a direct effect on the fluid permeability of the capillary walls.

The flow-enhancing effect of adrenaline was used to ensure the exposure of urine-forming capillaries to two inhibitors of active fluid transport, ouabain and 2,4-dinitrophenol (DNP). Both substances lowered the single glomerulus filtration fraction (SGFF), probably by affecting a fluid secretion mechanism. In addition, DNP diminished the flow-enhancing effect of adrenaline. This study provides relatively unequivocal evidence that fluid secretion underlies the formation of primary urine by the hagfish.

Key words: perfused glomerulus, adrenaline, inhibitor, colloid, hagfish, myxinoid, kidney, fluid secretion.

Introduction

When hagfish glomeruli are perfused with Ringer's solutions that contain colloid, a force that opposes pressure filtration, the effective colloid osmotic pressure (COP_{eff}), most of the time exceeds the force that favours pressure filtration, the hydrostatic pressure in the glomerular capillaries (P_{GC}). Despite this, urine is produced continuously (Riegel, 1998b). Some process other than pressure filtration underlies the formation of the primary urine in the hagfish.

The logical alternative to pressure filtration is fluid secretion, but evidence in support of that possibility is equivocal. Inhibitors of fluid-secreting tissues, 2,4-dinitrophenol and ouabain, depressed the single glomerulus filtration rate (SGFR) without causing detectable changes in the perfusion pressure (P_{perf}) (Riegel, 1978). From this it was surmised that the inhibitors lowered the fluid permeability of the urine-forming epithelium. However, it was found subsequently that putative stimulants of fluid secretion, such as theophylline, also depressed the SGFR (Riegel, 1998a). These observations were rationalised by the suggestion (Riegel, 1998b) that the glomerular vasculature of the hagfish may be made up of two flow pathways only one of which participates directly in urine formation. Inhibitors of fluid secretion may have the dual effect of (1) causing a shift of perfusate flow away from urine-forming vessels and (2) effecting a change in the fluid permeability of the glomerular vessels. Until recently, there seemed to be no possibility of discriminating between the two effects. However, in their study of the effects of adrenaline and noradrenaline on perfused glomeruli of *Myxine glutinosa*, Fels et al. (1987) found that the catecholamines elevated P_{perf}, SGFR and the single glomerulus filtration fraction (SGFF); these results could have been underlain by an elevation of pressure in the urine-forming capillaries because a colloid-free perfusate was used. Nevertheless, it seemed worthwhile to investigate the catecholamines for possible flow-enhancing properties. That investigation forms the basis of most of the research reported here.

Hagfish glomerular capillaries are relatively permeable to Ficoll 70: urine produced by glomeruli perfused with Ringer's solutions that contain Ficoll 70 usually has an appreciable COP (Riegel, 1978, 1986c, 1998a,b). Mesentarial capillaries of laboratory rats (Gamble, 1978) and frogs (Michel and Phillips, 1985) also are quite permeable to Ficoll 70; this permeability can be reduced by adding small amounts of bovine serum albumin (BSA) to perfusates. In the present study, the effects of BSA on the function of perfused glomeruli and on the appearance of colloid in the urine were studied.

Materials and methods

Specimens of a Pacific hagfish, *Eptatretus stouti* (Lockington), were studied at the marine laboratory of the University of California, Davis, in Bodega Bay, California,

USA. Animals were anaesthetised during experimentation, and they were not permitted to recover conciousness. Most methods used have been described in detail previously (Riegel, 1978, 1986b,c, 1998a), but they will be summarised briefly. A glomerulus was isolated from its afferent blood supply and perfused through a dual microcannula (Riegel, 1998b) tied into the segmental artery. The SGFR was measured by noting the rate of advance of urine into a liquid-paraffin-filled catheter tied into an isolated segment of the ureter adjacent to the perfused glomerulus. Only one glomerulus was perfused in each animal; the number of glomeruli (animals) studied in each series of perfusions is indicated in the text and in the tables by *N*.

Fluid delivery to perfused glomeruli was under the control of a dual-channel perfusion pump (Riegel, 1986c, 1998a). In most experiments, a uniform perfusion rate of 2.7 μl min^{-1} was used (Table 1B); this procedure minimised variations in the data and most of the time maintained P_{perf} below the upper limit of pressure (i.e. approximately 1.5 kPa) found in the dorsal aorta of intact specimens of *E. stouti*.

A number of perfusates were used: (1) hagfish Ringer's solution (Riegel, 1978) alone, which will be called 'plain Ringer'; (2) hagfish Ringer's solution to which either 0.1 % BSA (0.1 % BSA Ringer) or 0.5 % BSA (0.5 % BSA Ringer) and Ficoll 70 had been added; and (3) hagfish Ringer's solution to which 3 % BSA had been added (3 % BSA Ringer). Except for 'plain Ringer' and the perfusate for two experiments described below, the total COP of the perfusates (COP_p) was adjusted to 1.4 kPa, a value approximating the blood-plasma COP of *E. stouti*. In two experiments, glomeruli were perfused with 0.5 % BSA Ringer whose total COP was adjusted to 2.4 kPa by adding extra Ficoll 70. This was carried out to examine the effect on glomerular function of a value of COP_p well above that of the blood plasma of the experimental animal. Values of COP_p were calculated using the equations of Landis and Pappenheimer (1963) for BSA and Gamble (1983) for Ficoll 70; COP_p and the COP of the urine (COP_u) were measured periodically using methods described previously (Riegel, 1986a). Calculated and measured values of COP_p did not differ significantly.

P_{perf} was measured by blood-pressure transducers in the perfusion lines. Electrical output from the pressure transducers was amplified and led into an analogue-to-digital (A/D) converter (Mini-pod 100, Computer Instrumentation Ltd, Goring-by-Sea, UK) connected to a computer. Pressure readings and time notations were logged by a spreadsheet program (Excel 5, Microsoft Corporation) using software supplied with the A/D converter.

Single glomeruli were perfused for periods that varied from approximately 1.5 to 4 h. The following series of experiments were carried out.

Effects of various perfusates on P_{perf}, SGFR and COP_u

Perfusates that contained BSA

In one series of experiments, glomeruli were perfused continuously with 0.1 % BSA Ringer (*N*=11), 0.5 % BSA Ringer (*N*=7) or 3 % BSA Ringer (*N*=7). In a second series of experiments, glomeruli were perfused alternately with plain Ringer and one of 0.1 % BSA Ringer (*N*=4), 0.5 % BSA Ringer (*N*=5) or 3 % BSA Ringer (*N*=4). Experiments of the second series were made to determine the effects of BSA on P_{perf} and SGFR.

Perfusates that contained catecholamines and adrenergic receptor antagonists

Adrenaline and noradrenaline

To establish the minimum concentration of adrenaline that was effective in elevating P_{perf} and SGFR, glomeruli were perfused with a range of concentrations from 100 μmol l^{-1} (Fels et al., 1987) to 100 nmol l^{-1}; except for 100 nmol l^{-1} (*N*=3), all concentrations of adrenaline within this range affected P_{perf} and SGFR. However, the minimum concentration that consistently elevated P_{perf} and SGFR was 5 μmol l^{-1}; for that reason, 5 μmol l^{-1} was used as the standard concentration for adrenaline.

Noradrenaline, at a concentration of 5 μmol l^{-1}, affected P_{perf} and SGFR of perfused glomeruli in a manner indistinguishable from adrenaline except that its effects were not consistent. This lack of consistency caused noradrenaline to be rejected from further consideration as a possible flow-enhancing factor.

Adrenergic receptor (adrenoceptor) antagonists

To examine the likelihood that adrenaline acted by stimulating adrenergic receptors, phentolamine (an α-1-adrenoceptor antagonist) and propranolol (a β-adrenoceptor antagonist) were employed. Glomeruli were perfused continuously with Ringer's solution that contained either 1 μmol l^{-1} phentolamine (*N*=2) or 1 μmol l^{-1} propranolol (*N*=2). Glomeruli also were perfused initially with plain Ringer that contained 1 μmol l^{-1} phentolamine; after approximately 1 h, a switchover was made to plain Ringer that contained both 1 μmol l^{-1} phentolamine and 5 μmol l^{-1} adrenaline (*N*=6). Similarly, glomeruli (*N*=7) were perfused initially with plain Ringer that contained 1 μmol l^{-1} propranolol, followed by plain Ringer that contained both 1 μmol l^{-1} propranolol and 5 μmol l^{-1} adrenaline.

Perfusates that contained substances that reduced the SGFR

The apparent flow-enhancing property of 5 μmol l^{-1} adrenaline was utilised to study its influence on the effects of substances that normally diminish the SGFR of perfused glomeruli (Riegel, 1978); the substances tried were colloids (0.5 % BSA Ringer), an inhibitor of oxidative phosphorylation, 2,4-dinitrophenol (DNP), and the Na^+/K^+-ATPase inhibitor ouabain. Glomeruli were perfused initially with plain Ringer; after at least 1 h, a switchover was made to one of five test solutions: (1) plain Ringer + 5 μmol l^{-1} adrenaline (*N*=6); (2) plain Ringer + 5 μmol l^{-1} adrenaline + 100 μmol l^{-1} DNP (*N*=7); (3) plain Ringer + 5 μmol l^{-1} adrenaline + 10 μmol l^{-1} ouabain (*N*=6); (4) 0.5 % BSA Ringer (*N*=7); and (5) 0.5 % BSA Ringer + 5 μmol l^{-1} adrenaline (*N*=5).

Calculated parameters

Values of P_{perf} and the SGFR were divided by the perfusion rate prevailing during their measurement to yield the vascular resistance (P_{perf}/perfusion rate) and SGFF (SGFR/perfusion rate).

Reflexion coefficients (σ) for BSA and Ficoll 70 were calculated using the equation (Staverman, 1951):

$$\sigma = 1 - (C_u/C_p), \quad (1)$$

where C_u and C_p are, respectively, the concentration of colloid in the urine and in the perfusate. BSA and Ficoll 70 concentrations (g %) were calculated from COP measurements using the equations of, respectively, Landis and Pappenheimer (1963) and Gamble (1983). The effective COP (COP_{eff}) in perfused glomerular capillaries was calculated using the equation:

$$COP_{eff} = \sigma(COP_p - COP_u), \quad (2)$$

modified slightly from Michel (1997).

The slopes of the linear regressions relating vascular resistance to SGFF in Fig. 2 were all based on relatively large samples (N>30), so the significance of the slope could be determined in the usual way: the calculated slopes were divided by their standard errors, yielding values corresponding to the number of standard deviations from zero. In all cases, the values exceeded 1.96, so P<0.05. The significance of differences between the mean resistance or mean SGFF between control perfusions and perfusions with the various experimental solutions shown in Table 2 was tested by calculating the normal deviation (D) between mean values; the probability of the D values obtained was acquired from a standard table (Bailey, 1976). Values of the vascular resistance prevailing during periods of control perfusion for each glomerulus were averaged, and all values of vascular resistance calculated for that perfusion were divided by this value and multiplied by 100; this procedure transformed the data to a percentage of the average control value (i.e. % control average). Values of % control average prevailing during the control periods of all perfusions made using each of the experimental perfusates were averaged; similarly, values of % control average prevailing during the experimental periods of perfusion using each experimental perfusate were averaged. Differences between these means were tested statistically by calculating the value of D. Furthermore, because they had a common base, differences between means derived from perfusion with

Table 1. *Effects on the perfusion pressure, the single glomerulus filtration rate and the urinary colloid osmotic pressure when glomeruli were perfused continuously with various perfusates*

Perfusion rate (µl min^{-1})	Perfusion medium	SGFR (nl min^{-1})	P_{perf} (kPa)	COP_u (kPa)	N
A					
4.9±3.4 (129)	Ficoll Ringer	83.0±89.6	0.95±0.33	0.40±0.30	8
5.2±3.9 (91)	3 % BSA Ringer	*45.7±45.0*	*1.15±0.30*	*0.16±0.26*	7
4.8±3.5 (220)	0.1 % BSA Ringer	80.6±89.2	0.96±0.41	0.30±0.30	11
B					
2.7 (65)	0.1 % BSA Ringer	83.0±89.1	0.88±0.17	0.30±0.30	11
2.7 (31)	0.5 % BSA Ringer	93.8±57.8	0.97±0.17	0.36±0.25	7
2.7 (42)	3 % BSA Ringer	*52.2±38.5*	1.00±0.29	*0.16±0.26*	7
C					
2.7 (50)	Plain Ringer	**420±188**	**0.68±0.17**	0.0098±0.0003	8
2.7 (55)	Plain Ringer + Adr	**572±216**	**0.85±0.30**	0.0053±0.0076	13

Numbers in parentheses following the perfusion rate are the number of determinations of the perfusion pressure (P_{perf}) and the single glomerulus filtration rate (SGFR).

N is the number of glomeruli perfused.

The data for perfusion rates in A and for SGFR and P_{perf} in A, B and C were analysed for the significance of the differences between the means using Student's t-test for large samples and assuming the data to have a normal distribution.

Mean values for the same variable in each section of the table that are shown in normal type do not differ significantly.

Mean values of each parameter in each section of the table that are shown in italic type differ significantly (P<0.05) from mean values of the same variable in the same section that are shown in normal type.

Mean values in bold type differ significantly (P<0.05) from mean values of the same variable also shown in bold type.

A, comparison of Ficoll Ringer (data from Riegel, 1998a,b) with 3 % BSA Ringer and 0.1 % BSA Ringer.

B, comparison of 0.1 % BSA Ringer, 0.5 % BSA Ringer and 3 % BSA Ringer when glomeruli were perfused at the same rate.

C, comparison of plain Ringer (no colloid) with plain Ringer that contained 5 µmol l^{-1} adrenaline when glomeruli were perfused at the same rate.

Values are shown as means ± S.D.

The urinary colloid osmotic pressure (COP_u) of all perfusion media that contained colloid was 1.4 kPa.

Adr, adrenaline.

different experimental solutions could also be tested statistically.

Results

Effects of colloid on perfused glomeruli

Glomeruli perfused continuously with Ringer's solutions that contained BSA

Table 1A shows mean values of SGFR, P_{perf} and COP_u for glomeruli perfused continuously with 0.1 % BSA Ringer and 3 % BSA Ringer. Results of similar experiments in which glomeruli were perfused with Ringer's solution that contained only Ficoll 70 (Ficoll Ringer) taken from Riegel (1998a,b) are included for comparative purposes. There were no significant differences between mean values of SGFR, P_{perf} or COP_u of glomeruli perfused with either 0.1 % BSA Ringer or Ficoll Ringer. Addition of a small amount of BSA to perfusates that contained Ficoll 70 did not significantly reduce the entry of colloid into the urine. However, complete replacement of Ficoll 70 with BSA in the perfusates significantly lowered SGFR and COP_u and elevated P_{perf} of the perfused glomeruli. It should be noted that the averaging of the data in Table 1A,B obscured the fact that, in five of the seven perfusions involving 3 % BSA Ringer, COP_u was negligible (<0.08 kPa).

Glomeruli perfused continuously at a uniform rate with Ringer's solutions that contained BSA

As shown in Table 1B, there were no significant differences between the mean values of P_{perf} during perfusion by any of the perfusates. However, mean values of SGFR and COP_u of glomeruli perfused with 3 % BSA Ringer were significantly lower than mean values of SGFR and COP_u of glomeruli perfused with either 0.1 % BSA Ringer or 0.5 % BSA Ringer.

Glomeruli perfused alternately with plain Ringer and Ringer's solutions that contained BSA

Alternating plain Ringer and solutions containing colloid had similar effects on P_{perf} and SGFR irrespective of the colloid used: an experiment with 3 % BSA Ringer will be used as an illustration. As shown in Fig. 1A, a glomerulus was perfused at 2.7 μl min^{-1} alternately with plain Ringer and 3 % BSA Ringer. When 3 % BSA Ringer replaced plain Ringer as the perfusate, P_{perf} became elevated and SGFR became depressed. When the preparation was once again perfused with plain Ringer (at approximately 28 min), P_{perf} remained elevated. This effect was seen commonly in single hagfish glomeruli perfused with colloidal solutions.

One of two experiments in which glomeruli were perfused at 2.7 μl min^{-1} alternately with plain Ringer and with 0.5 %

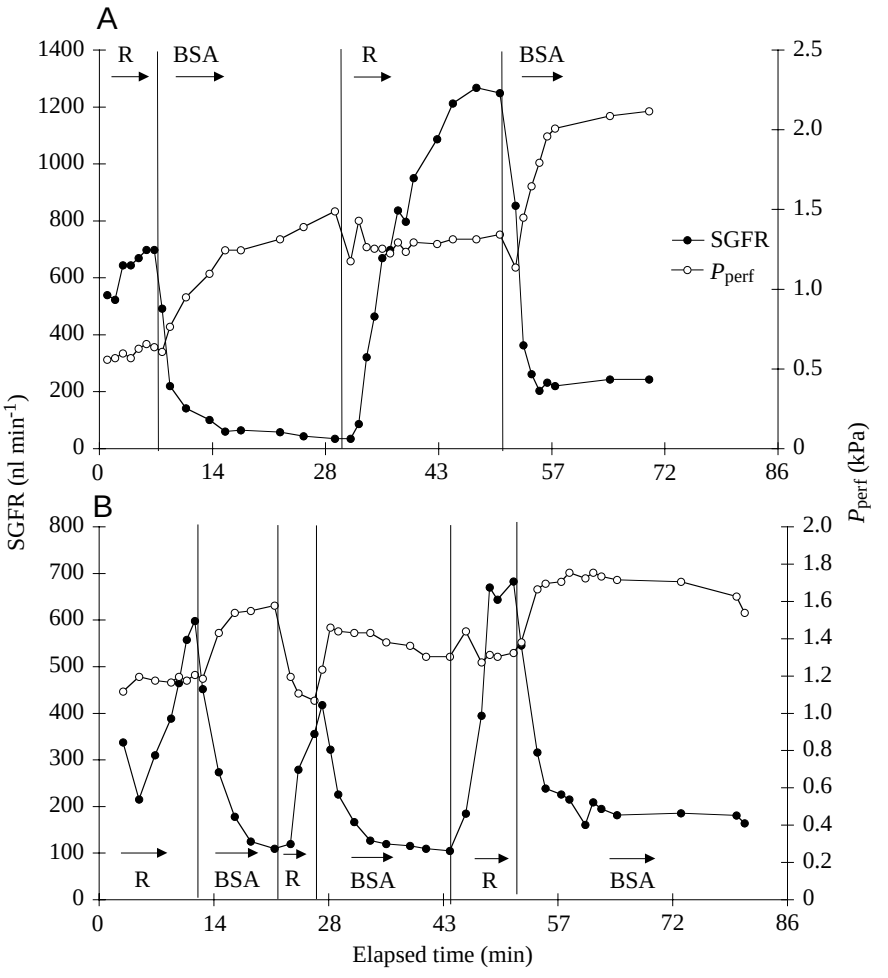

Fig. 1. Changes in the single glomerulus filtration rate (SGFR) and the perfusion pressure (P_{perf}) that occurred when a glomerulus was perfused alternately with plain Ringer (R) and with either (A) 3 % BSA Ringer or (B) 0.5 % BSA Ringer (BSA) in which the perfusate colloid osmotic pressure (COP_p) had been increased to 2.41 kPa. Vertical solid lines on the figure indicate where the perfusion medium was changed.

BSA Ringer whose COP_p was increased to a value (2.41 kPa) well in excess of the plasma COP of *E. stouti* is shown in Fig. 1B. Comparison of Fig. 1B and Fig. 1A reveals no obvious differences in relative changes in P_{perf} and SGFR, so clearly these variables were not differentially affected by a COP_p well above normal.

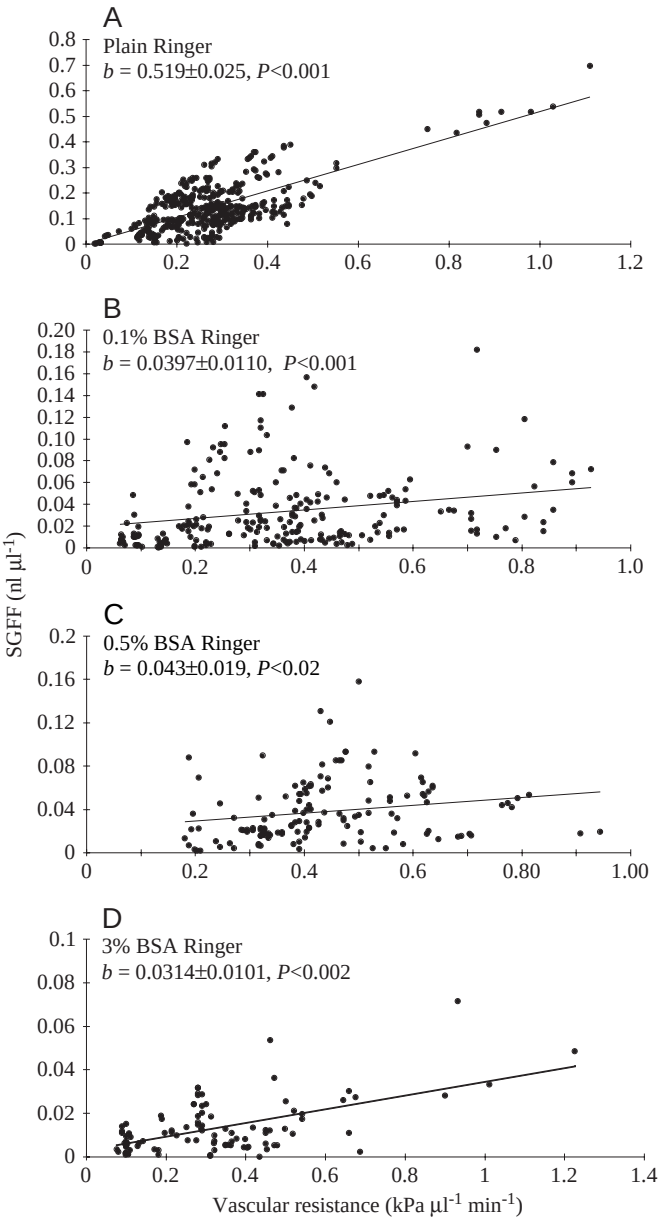

Fig. 2. The effect of various perfusates on the relationship between the vascular resistance and the single glomerulus filtration fraction (SGFF) of perfused glomeruli. Equations for the regression lines were: (A) plain Ringer, y=0.5187x+0.0005; (B) 0.1 % BSA Ringer, y=0.0397x+0.0110; (C) 0.5 % BSA Ringer, y=0.043x+0.028; and (D) 3 %BSA Ringer, y=0.0314x+0.0032. The linear regression lines were fitted by the method of least squares outlined by Bailey (1976). As indicated by their regression coefficients, the positive slopes of all the regression lines are significantly different from zero (P<0.05).

Vascular resistance and SGFF

Fig. 2A–D shows plots of values of the vascular resistance and SGFF calculated from the data obtained when glomeruli were perfused continuously with either plain Ringer or Ringer's solutions that contained BSA. As shown by the slopes of the linear regression lines, there was a consistent and significant positive relationship between vascular resistance and SGFF. It is likely that the steepness of the slope of the regression line calculated for data derived from plain Ringer perfusions reflects the fact that pressure filtration was the major contributor to the SGFF.

Effects of adrenaline and adrenoceptor antagonists

Adrenaline

As shown in Fig. 3, when a glomerulus was perfused with plain Ringer followed by plain Ringer + 5 µmol l^{-1} adrenaline, P_{perf} was elevated from approximately 0.8 kPa to a maximum of approximately 1.4 kPa. There was a concomitant elevation of SGFR. When perfusion with plain Ringer was recommenced, P_{perf} returned to values close to those seen prior to adrenaline perfusion. Data for all glomeruli (N=13) perfused with plain Ringer + 5 µmol l^{-1} adrenaline are summarised in Table 1C. It can be seen that adrenaline caused significant increases in the mean values of both SGFR (by approximately 36 %) and P_{perf} (by approximately 25 %).

Adrenoceptor antagonists

Neither P_{perf} nor SGFR was noticeably altered when glomeruli were perfused continuously with plain Ringer that contained either 1 µmol l^{-1} phentolamine or 1 µmol l^{-1} propranolol. However, when glomeruli where perfused with plain Ringer that contained 1 µmol l^{-1} phentolamine + 5 µmol l^{-1} adrenaline (N=6), elevation of P_{perf} and SGFR did not occur. The effect of 1 µmol l^{-1} phentolamine is illustrated in Fig. 4. The effect of propranolol on the responses to adrenaline was not consistent. In three of seven perfusions, 1 µmol l^{-1} propranolol had little effect on the elevation of P_{perf} and SGFR by 5 µmol l^{-1} adrenaline; in four perfusions, the action of adrenaline was blocked.

Effects of DNP and ouabain

Table 2 summarises the effects of various perturbants of the vascular resistance and the SGFF of perfused hagfish glomeruli. Mean values of vascular resistance and SGFF are given and analysed statistically; to make comparisons easier, averaged values of the percentage change in the mean values of these variables are given also. Perfusion of glomeruli with plain Ringer + 5 µmol l^{-1} adrenaline caused significant increases in the mean values of both vascular resistance and SGFF (Table 2A, section 1). However, 100 µmol l^{-1} DNP appeared to block the action of adrenaline in elevating vascular resistance, whilst significantly reducing the SGFF of perfused glomeruli (Table 2A, section 2). The mean vascular resistance of glomeruli perfused with plain Ringer + 5 µmol l^{-1} adrenaline was unaffected by 10 µmol l^{-1} ouabain, but mean values of SGFF were significantly reduced (Table 2A, section 3).

Table 2. *Changes in the vascular resistance and the single glomerulus filtration fraction of single glomeruli perfused initially with plain Ringer, followed by one of five experimental solutions*

	Resistance		SGFF	
	(kPa µl^{-1} min^{-1})	(% control average)	(nl µl^{-1})	(% control average)
A				
(1) Plain Ringer + 5 µmol l^{-1} adrenaline (six glomeruli)				
Control (50)	0.21±0.01	100±1.0	0.1156±0.0102	100±3.1
Experimental (53)	**0.28±0.02**	**124±3.3**[a]	**0.1760±0.0140**	**125±4.5**
(2) 5 µmol l^{-1} adrenaline + 100 µmol l^{-1} 2,4-dinitrophenol (seven glomeruli)				
Control (37)	0.26±0.01	100±1.8	0.0901±0.0062	100±2.2
Experimental (36)	0.26±0.01	99.7±2.1[a]	**0.0481±0.0035**	**58.9±1.6**
(3) 5 µmol l^{-1} adrenaline + 10 µmol l^{-1} ouabain (six glomeruli)				
Control (34)	0.26±0.01	100±1.2	0.1317±0.0073	100±1.6
Experimental (36)	**0.36±0.03**	**123±5.1**	**0.1025±0.0060**	**82.1±2.8**
B				
(1) 0.5 % BSA Ringer (seven glomeruli)				
Control (38)	0.31±0.02	100±1.1	0.1322±0.0098	100±4.0
Experimental (40)	**0.42±0.03**	**128±1.6**[b]	**0.0401±0.0048**	**27.6±2.6**[c]
(2) 0.5 % BSA Ringer + 5 µmol l^{-1} adrenaline (five glomeruli)				
Control (27)	0.30±0.01	100±1.5	0.1752±0.0174	100±1.8
Experimental (36)	**0.48±0.01**	**154±4.9**[b]	**0.0828±0.0074**	**43.0±2.5**[c]

Values shown (mean ± S.E.M.) are either those calculated directly from measurements of perfusion pressure (P_{perf}) and single glomerulus filtration rate (SGFR) (i.e. resistance = kPa µl^{-1} min^{-1}; SGFF = nl µl^{-1}) or represent means of percentage-transformed data (% control average; see Materials and methods).

The number of glomeruli perfused is indicated in parentheses following descriptions of the experimental perfusates.

The number of variates used to calculate mean values of either control or experimental values are indicated by the numbers in parentheses after the words 'control' and 'experimental'.

The statistical significance of the differences between the mean values of vascular resistance and single glomerulus filtration fraction (SGFF) during control and experimental periods of perfusion was determined as follows. The value of the normal variation (D) was calculated and its probability was taken from a standard table (Bailey, 1976).

Mean values in bold type for experimental perfusions differ significantly (P<0.05) from corresponding mean values during control perfusion.

Values marked with similar superscript letters indicate mean values that are statistically significantly different from each other (P<0.05).

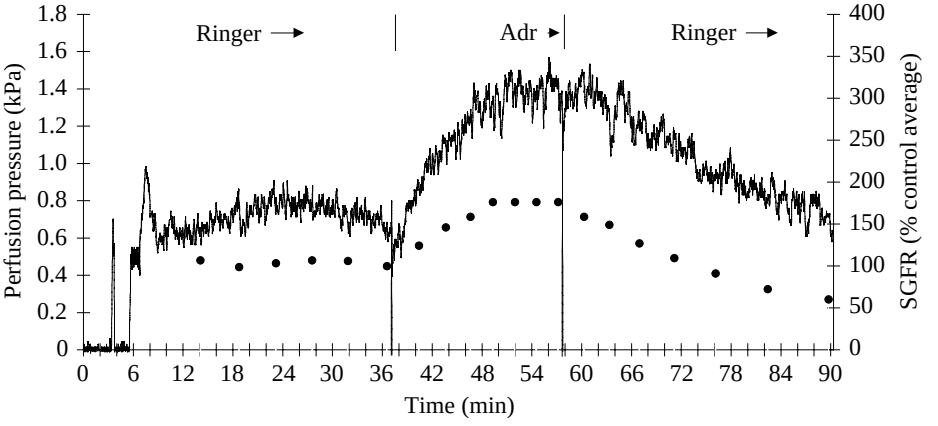

Fig. 3. The effect of adrenaline on the perfusion pressure (P_{perf}) and the single glomerulus filtration rate (SGFR) of a glomerulus perfused alternately with plain Ringer and plain Ringer + 5 µmol l^{-1} adrenaline (Adr). The SGFR (filled circles) was calculated as a percentage of its mean value during the initial perfusion with plain Ringer (=% control average).

The vascular resistance of perfused glomeruli was significantly increased by 0.5 % BSA Ringer, whilst SGFF was considerably reduced by that colloid (Table 2B, section 1). When 5 µmol l^{-1} adrenaline was added to the perfusate, both the vascular resistance and SGFF increased significantly relative to their values during perfusion with 0.5 % BSA Ringer alone (Table 2B, section 2).

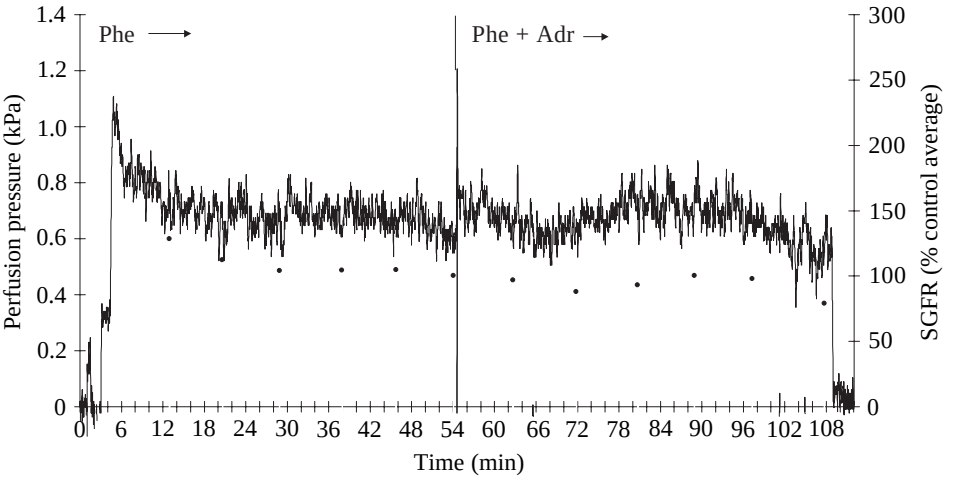

Fig. 4. The effect of 1 μmol l^{-1} phentolamine on the elevation of the perfusion pressure (P_{perf}) and the single glomerulus filtration rate (SGFR) by adrenaline. The glomerulus was perfused initially with plain Ringer + 1 μmol l^{-1} phentolamine (Phe), and then a switchover was made to perfusion with plain Ringer + 1 μmol l^{-1} phentolamine + 5 μmol l^{-1} adrenaline (Phe+Adr). The SGFR (filled circles) was calculated as a percentage of its mean value during the initial perfusion with plain Ringer (=% control average).

Discussion

Effects of colloid on perfused hagfish glomeruli

It is clear from the data shown in Fig. 1 and in Table 1 that the presence of colloid considerably diminishes urine formation by perfused hagfish glomeruli. However, this effect does not seem to be due entirely to the osmotic pressure exerted by the colloid. This can be illustrated by two experiments: in the first (Fig. 1A), glomeruli were perfused with a Ringer's solution in which the colloid concentration approximated that of normal plasma; in the second (Fig. 1B), glomeruli were perfused with Ringer's solution in which the colloid concentration was well above the normal plasma COP. The measured COP of the urine produced during the experiment illustrated in Fig. 1A was negligible (a common occurrence when glomeruli were perfused with 3 % BSA Ringer: see the discussion of the results shown in Table 1A,B). Approximately 25 % of the total urine volume produced during the experiment was produced during periods when the glomerulus was perfused with 3 % BSA Ringer. Had more than an insignificant amount of colloid entered the urine during that time, it would have been measurable (Riegel, 1986c). Since the COP_u in the experiment was zero, the reflection coefficient σ of BSA was 1 (see Materials and methods); therefore, the COP_{eff} of the perfusate was equal to the COP_p (i.e. approximately 1.4 kPa). At times, P_{perf} was equal to or exceeded COP_{eff}, but it is unlikely that P_{GC} would have been adequate to effect pressure filtration (Riegel, 1998b) except, possibly, near the end of the experiment.

The experiment shown in Fig. 1B illustrates unequivocally the insensitivity to COP of the process of urine formation in the hagfish. The measured COP_u was approximately 0.04 kPa. Approximately equal quantities of urine were produced during perfusion with plain Ringer (COP_u=0, Table 1C) and during perfusion with 0.5 % BSA Ringer (COP_u>0, Table 1B). Therefore, the maximum COP_u during perfusion of colloid-containing Ringer could have been no more than approximately 0.08 kPa. The reflection coefficient σ of Ficoll 70 calculated for the experiment was 0.95, so the COP_{eff} of the perfusate was approximately 2.2 kPa. There is no possibility that P_{GC} could have exceeded COP_{eff}, since the maximum value of P_{perf} was only approximately 1.8 kPa.

During experiments of the kind illustrated in Fig. 1, P_{perf} usually became considerably elevated after the switchover from plain Ringer to colloid-containing Ringer. This effect can be seen in Fig. 1A at approximately 54 min and in Fig. 1B every time perfusion was switched from plain Ringer to 0.5 % BSA Ringer. Since the glomeruli were perfused at a constant rate, the increases in P_{perf} indicated that the vascular resistance had increased. The evidence is persuasive (see below) that the vascular resistance of perfused hagfish glomeruli changes with the same polarity as the area of the urine-forming surfaces. It could be argued that, in perfusions such as those illustrated in Fig. 1, colloid caused the area of the urine-forming surfaces to increase; therefore, P_{perf} remained elevated after perfusion with plain Ringer was recommenced because the area of the urine-forming surfaces remained expanded. This could provide a partial explanation of why there was such a rapid elevation, and often an overshoot, of SGFR when switching from colloid-containing Ringer to plain Ringer (e.g. Fig. 1A after 28 min, Fig. 1B after 43 min). Possibly colloid causes increases in flow in the urine-forming capillaries of the hagfish glomerulus. It should be said that this contention runs counter to the argument usually advanced to explain the effects of colloid on urine formation, namely, that colloid reduces the flow in the glomerular capillaries (see Riegel, 1978).

Factors contributing to primary urine formation in hagfish

Filtration of fluid across a porous surface is underlain by three factors: the area and fluid permeability of the surface and the force that causes fluid to move. The nature of the force causing transepithelial fluid movement in hagfish glomeruli is unknown, but it appears to be controlled by the rate of fluid delivery to the urine-forming epithelia (Stolte and Eisenbach, 1973; Riegel, 1978, 1986c,1998a,b, present study; Fels et al., 1993). Therefore, when the rate of fluid delivery is held constant, it would be expected that variations in the fluid permeability and the area of the urine-forming surfaces would

determine the rate of urine formation. That premise underlies the following discussion.

Analysis of factors effecting changes of area and fluid permeability

Table 2 shows the effects of perturbants of SGFR that appeared to have differential effects on the fluid permeability (colloid, ouabain, DNP) and the area (adrenaline) of the urine-forming surfaces. In discussing the results summarised in Table 2, changes in the vascular resistance will be utilised to estimate changes in the area of urine-forming surfaces; changes in SGFF relative to changes in the vascular resistance will be utilised to estimate changes in the fluid permeability of the urine-forming surfaces.

The key results of these experiments are shown in Table 2A (section 1) and Table 2B (sections 1 and 2): when the perfusate contained either colloid or adrenaline, the vascular resistance of the glomeruli was increased, indicating that the area of the urine-forming surfaces had increased. The presence of colloid reduced SGFF, probably because of a reduction in the fluid permeability; this effect of colloid has been demonstrated in other perfused preparations (e.g. Mason et al., 1977; Fried et al., 1986). During perfusion with plain Ringer + adrenaline, pressure filtration was possible, so the increase in SGFF was probably underlain by that process. When both colloid and adrenaline were perfused (Table 2B, section 2), the vascular resistance and SGFF were elevated significantly above the values resulting from perfusion with colloid alone (Table 2B, section 1). This result must have been underlain entirely by an increase in the area of the urine-forming surfaces.

Actions of inhibitors of fluid secretion

When glomeruli were perfused with plain Ringer + DNP + adrenaline, there was no apparent change in the vascular resistance, but there was a reduction in SGFF of approximately 40 % (Table 2A, section 2). This result suggested that DNP had a direct effect on the urine-forming epithelia. During perfusion with plain Ringer, it would be expected that changes in the vascular resistance and SGFF would be of the same polarity due to pressure filtration. Since adrenaline did not elevate the vascular resistance, it is likely that DNP also had an effect on the mechanism that causes flow changes in hagfish glomeruli.

When glomeruli were perfused with plain Ringer + ouabain + adrenaline, the vascular resistance was increased and SGFF was reduced (Table 2A, section 3); it is likely that ouabain, like DNP, had a direct effect on the urine-forming epithelia. Ouabain depressed SGFF by only approximately 20 %, suggesting that the decrease in fluid permeability of the urine-forming surfaces was overcome by pressure filtration. The increase in the vascular resistance provoked by adrenaline was not different from that of controls (Table 2A, section 1). Therefore, it appeared that ouabain had no effect on the mechanism that controls flow changes in perfused glomeruli.

The data shown in Table 2 and analysed above provide compelling evidence that well-characterised inhibitors of fluid secretion also act in that capacity in perfused hagfish glomeruli.

The efficiency of fluid secretion by hagfish glomeruli

The insensitivity of transepithelial fluid movement to COP seen in perfused hagfish glomeruli has also been reported in other fluid-secreting tissues, for example, the Malpighian tubules of the blood-sucking bug *Rhodnius prolixus* (Stål). Malpighian tubules of *R. prolixus* secrete fluid at one of the fastest rates known: $50\ \mathrm{nl\ s^{-1}\ cm^{-2}}$ (Maddrell, 1991, S. H. P. Maddrell, personal communication). Despite their high fluid permeability, the Malpighian tubules of *R. prolixus* have a low passive permeability to solutes: small organic molecules (<500 Da) enter the secreted fluid at a concentration usually much less than 10 % of their concentration in the bathing medium. Inulin (molecular mass ≈ 5 kDa) enters the secreted fluid at a concentration of only approximately 0.8 % of the concentration in the medium (Maddrell and Gardiner, 1974). In contrast, the urine-forming epithelia of hagfish glomeruli have a high passive permeability to both fluid and solutes: the area-related fluid permeability of glomeruli perfused with plain Ringer was calculated to be $159\ \mathrm{nl\ s^{-1}\ cm^{-2}}$. [Rates of fluid permeability related to glomerular surface area were calculated from the maximum value of SGFR shown in Table 1C and the minimum value of SGFR shown in Table 1B; the area of hagfish glomerular capillaries (approximately $6\ \mathrm{mm^2}$) was taken from measurements made by Fels et al. (1993).] An appreciable proportion of perfused Ficoll 70 (molecular mass ≈ 67 kDa) usually appears in the urine (Table 1A), and the glomerular capillaries are freely permeable to inulin (Munz and McFarland, 1963).

Studies by Echlin et al. (1977) have shown that fluid secretion by the Malpighian tubules of *R. prolixus* was little affected by colloid (dextran) whose COP was approximately 3 kPa. The low passive permeability of *R. prolixus* Malpighian tubules greatly increased the effectiveness of the fluid-secretion mechanism; in contrast, the volume of primary urine formed by hagfish glomeruli is probably reduced by a large simultaneous backflow due to non-penetrating colloid. The magnitude of this reduction can be appreciated by comparing the value of the maximum area-related fluid permeability cited above ($159\ \mathrm{nl\ s^{-1}\ cm^{-2}}$) with the area-related fluid permeability calculated (see above) for glomeruli perfused with 3 % BSA Ringer, namely $14.5\ \mathrm{nl\ s^{-1}\ cm^{-2}}$.

Although fluid secretion by epithelia as permeable as are those of the hagfish glomerulus would appear to be energetically inefficient, the high passive permeability would ensure rapid movement of solutes from the blood to the urine. Perhaps, in the evolution of the hagfish kidney, the advantages of forming primary urine by a relatively unselective process overrode the possible disadvantage of a high energy cost. Alternatively, it is possible that fluid secretion may have been imposed upon the hagfish because of the low systemic hydrostatic pressures to which its metabolic processes must have had to adapt.

I thank the Director, Dr J. S. Clegg, and the staff of the Bodega Marine Laboratory for much help during this study. I am grateful to Professors Stefan Nilsson and Charles Michel for helpful advice and to Dr Simon Maddrell for helpful discussions on questions of transepithelial fluid movement.

References

Bailey, N. T. J. (1976). *Statistical Methods in Biology*. *Biological Science Tests* (ed. D. R. Arthur and W. S. Bullough). London: Hodder & Stoughton.

Echlin, P., Skaer, H. le B., Gardiner, B. O. C., Franks, F. and Asquith, M. H. (1977). Polymeric cryoprotectants in the preservation of biological ultrastructure. II. Physiological effects. *J. Microsc*. **110**, 239–255.

Fels, L. M., Elger, B. and Stolte, H. (1987). Effects of catecholamines on the function of single glomeruli of *Myxine glutinosa* L. (Cyclostomata). In *Proceedings of the German Zoological Society, 80th Meeting in Ulm* (ed. F. G. Barth and E.-A. Seyfarth), p. 162. Stuttgart, New York: Gustav Fischer Verlag.

Fels, L. M., Sanz-Altamira, P., Decker, B., Elger, B. and Stolte, H. (1993). Filtration characteristics of the single isolated perfused glomerulus of *Myxine glutinosa*. *Renal Physiol. Biochem*. **16**, 276–284.

Fried, T. A., McCoy, R. N., Osgood, R. W. and Stein, J. H. (1986). Effect of albumin on glomerular ultrafiltration coefficient in isolated perfused dog glomerulus. *Am. J. Physiol*. **250**, F901–F906.

Gamble, J. (1978). The effect of low concentrations of bovine albumin on the vascular transudation coefficient of the isolated perfused rat mesentary. *J. Physiol., Lond.* **289**, 63P–64P.

Gamble, J. (1983). The influence of pH on capillary filtration coefficient of rat mesentaries perfused with solutions containing albumin. *J. Physiol., Lond*. **339**, 505–518.

Landis, E. M. and Pappenheimer, J. R. (1963). Exchange of substances through the capillary walls. In *Handbook of Physiology*, section 2, *Circulation*, vol. 2 (ed. W. F. Hamilton and P. Dow), pp. 961–1034. Washington, DC: American Physiological Society.

Maddrell, S. H. P. (1991). The fastest fluid-secreting cell known: the upper Malpighian tubule cell of *Rhodnius*. *BioEssays* **13**, 357–362.

Maddrell, S. H. P. and Gardiner, B. O. C. (1974). The passive permeability of insect Malpighian tubules to organic solutes. *J. Exp. Biol*. **60**, 641–652.

Mason, J. C., Curry, F. E. and Michel, C. C. (1977). The effects of proteins upon the filtration coefficient of individually perfused frog mesenteric capillaries. *Microvasc. Res*. **13**, 185–202.

Michel, C. C. (1997). Starling: the formulation of his hypothesis of microvascular fluid exchange and its significance after 100 years. *Exp. Physiol*. **82**, 1–30.

Michel, C. C. and Phillips, M. E. (1985). The effects of bovine serum albumin and a form of cationised ferritin upon the molecular selectivity of the walls of single frog capillaries. *Microvasc. Res*. **29**, 190–203.

Munz, F. W. and McFarland, W. N. (1963). Regulatory function of a primitive vertebrate kidney. *Comp. Biochem. Physiol*. **13**, 381–400.

Riegel, J. A. (1978). Factors affecting glomerular function in the Pacific hagfish *Eptatretus stouti* (Lockington). *J. Exp. Biol*. **73**, 261–277.

Riegel, J. A. (1986a). Measurement of colloid osmotic pressure of microlitre and sub-microlitre volumes. *J. Exp. Biol*. **122**, 433–437.

Riegel, J. A. (1986b). Hydrostatic pressures in glomeruli and renal vasculature of the hagfish *Eptatretus stouti*. *J. Exp. Biol*. **123**, 359–371.

Riegel, J. A. (1986c). The absence of arterial pressure effect on filtration by perfused glomeruli of the hagfish *Eptatretus stouti* (Lockington). *J. Exp. Biol*. **126**, 361–374.

Riegel, J. A. (1998a). An analysis of function of the glomeruli of the hagfish mesonephric kidney. In *The Biology of Hagfishes* (ed. J. M. Jørgensen, J.-P. Lomholt, R. Weber and H. Malte), pp. 364–377. London: Chapman & Hall.

Riegel, J. A. (1998b). Analysis of fluid dynamics in perfused glomeruli of the hagfish *Eptatretus stouti* (Lockington). *J. Exp. Biol*. **201**, 3097–3104.

Staverman, A. J. (1951). The theory of the measurement of osmotic pressure. *Recl. Trav. Chim. Pays Bas* **70**, 344–352.

Stolte, H. and Eisenbach, G. M. (1973). Single nephron filtration rate in the hagfish *Myxine glutinosa* (L.). *Bull. Mt Desert Island Biol. Lab*. **13**, 120–121.