Journal of Physiology (1995), **484.1**, *pp.* 183–187

Subatmospheric closing pressures in individual microvessels of rats and frogs

P. J. MacPhee and C. C. Michel

Department of Physiology and Biophysics, St Mary's Hospital Medical School, Imperial College of Science, Technology and Medicine, Norfolk Place, London W2 1PG, UK

1. We have investigated the hypothesis that ascending vasa recta (AVR) in the rat renal medulla are able to remain open when the external pressure is greater than the internal.

2. Individual vasa recta were cannulated in anaesthetized rats with Evans Blue albumin solution and then occluded downstream prior to the first branchpoint. When the intraluminal pressure was lowered, the lumina collapsed at a mean pressure of approximately $-4{\cdot}0$ cmH_2O for both AVR and descending vasa recta.

3. The studies were extended to include microvessels from rat spinotrapezius muscle and mesentery and frog mesentery; mean closing pressures were $-3{\cdot}2$, $-4{\cdot}2$ and $-5{\cdot}3$ cmH_2O, respectively.

4. Subatmospheric closing pressures may allow small differences in hydrostatic pressure alone to drive fluid uptake into the AVR.

During an investigation of the transport properties of the renal medullary microvessels, the question arose as to whether the vasa recta can remain open when the pressure inside them is less than that of the surrounding interstitial fluid. If so, the uptake of fluid into the ascending vasa recta (AVR) could be driven by a hydrostatic pressure gradient.

It is usually assumed that a vessel will collapse when the hydrostatic pressure outside is greater than that inside. In most tissues, fluid movement from tissue to blood is driven by oncotic pressure gradients in accordance with Starling's principle (1896), a process that is self-limiting wherever there is a finite permeability to protein (Michel, 1984). In the gastrointestinal tract and the cortex of the kidney, where the epithelial secretion of large volumes of protein-free fluid dilute the interstitial fluid, steady reabsorption into the blood vessels can occur as long as lymphatics are available to drain excess fluid and protein. Aukland, Bogusky & Renkin (1994) have recently developed this hypothesis for uptake of fluid by renal cortical capillaries under normal conditions.

In the renal medulla, however, where significant amounts of water are reabsorbed during urine concentration, the situation is more complex. Lymphatics have been demonstrated to descend as far as the outer medulla (Cuttino, Jennette, Clark & Kwock, 1985) but not into the inner medulla. This lack of drainage, coupled with considerable fluid movement into the interstitium from the collecting ducts during urine concentration, means that unless the ascending vasa recta (AVR) are impermeable to protein, there will be a tendency for protein concentrations in the interstitium to approach those in the plasma, reducing the oncotic pressure available for reabsorption. It is known, however, that plasma proteins are present in the renal medullary interstitium (Lassen, Longley & Lilienfield, 1958). Furthermore, we (MacPhee & Michel, 1993, 1995) have shown that, in fact, the reflection coefficient (σ) for albumin in the AVR is between 0·6 and 0·7. Similar estimates for σ (0·7) had previously been obtained by Pallone (1992). Thus, the AVR are permeable to serum albumin and presumably to other plasma proteins. Given the assumptions of an interstitial space without draining lymphatics (in the inner medulla) and AVR with a significant permeability to protein, an alternative hypothesis is necessary to account for steady fluid reabsorption by the AVR.

One possibility is a hydrostatic pressure gradient in favour of reabsorption. If this is to be a viable hypothesis, the AVR must be able to remain open when the external pressure is higher than the internal. Starling, in 1896, argued from histological observations of oedematous tissues that capillaries were surrounded and supported by an 'adventitia of radiating fibres' which might act as wires tethering the vessel in such a way as to keep the lumen open when interstitial fluid pressure exceeded

This manuscript was accepted as a Short Paper for rapid publication.

capillary pressure. Somewhat later, Burton and his colleagues examined vascular networks, for example those of the frog mesentery (Burton, 1951; Nichol, Girling, Jerrand, Claxton & Burton, 1951) and observed a sudden cessation of flow through the network when perfusion pressures fell to a critical, but still positive, value. They termed the pressure the critical closing pressure (CCP). They suggested that such closure was a fundamental consequence of the elastic properties of small vessels. CCPs have also been demonstrated in large vessels (Burton, 1951; van Dijk, Krams, Sipkema & Westerhof, 1988; Shrier, Hussain & Magder, 1993).

In the experiments reported here, we have examined whether or not the AVR are able to withstand a subatmospheric intraluminal pressure *in vivo*, raising the possibility of a hydrostatic pressure as a possible driving force for reabsorption in the renal medulla. To answer the more general question of whether individual (as opposed to networks of) small vessels are stable at subatmospheric intraluminal pressures, we have determined the closing pressures of microvessels from three organs (kidney, mesentery and muscle) and from two species (frog and rat).

A preliminary report of our results to The Physiological Society has been published (MacPhee & Michel, 1992).

METHODS

Studies performed on the vasa recta and on the venules of spinotrapezieus muscle were carried out on 2-week-old Sprague–Dawley rats, anaesthetized with a subcutaneous injection of Hypnorm (fentanyl citrate (0·315 mg ml^{-1}) and fluanisone (10 mg ml^{-1}), used at 1–3 ml kg^{-1}) and Hypnovel (midazolam hydrochloride, 5 ml kg^{-1} of 5 mg ml^{-1}). Body temperature was monitored by a rectal probe and maintained using a heating table. To obtain access to the vasa recta, the left kidney was approached via a lateral abdominal incision and supported in a holder to reduce respiratory movement. The papilla of the kidney was exposed by removal of the ureter. Intravascular pressures and microvascular closing pressures were determined for both ascending (AVR) and descending vasa recta (DVR). Experimental animals were killed by an overdose of the above anaesthetic mixture.

The spinotrapezius muscle was exposed using a mid-line incision of the skin of the dorsal surface of the rat. The muscle was separated from underlying muscles by gentle dissection and the superficial fascia was carefully removed with fine forceps. The muscle was positioned over a fibre-optic light source by means of several sutures attached to the edge of the muscle, and the surface kept moist with a modified Ringer solution (in g l^{-1}: 6·9 NaCl, 0·35 KCl, 0·1096 $MgCl_2$, 2·1 $NaHCO_3$, 0·06 NaH_2PO_4, 1·0 glucose, and 1·4 ml of a 20% w/v solution of $CaCl_2$). Small (first-order) venules were then cannulated with sharpened glass micropipettes with a tip diameter of 5–10 μm. Nerves and blood vessels were all intact, and located normally *in situ*.

Mesenteric capillaries were studied in frogs (*Rana temporaria*), which were stunned before the brain and upper spinal cord were destroyed by pithing. A loop of intestine was exteriorized through a lateral abdominal incision, draped over a Perspex pillar and kept moist with frog Ringer solution bubbled with 97% O_2, 3% CO_2. Capillaries were identified by flow characteristics, i.e. small vessels divergent from a supplying arteriole and located prior to convergence into draining venules. All the capillaries were perfused with 5% bovine serum albumin (BSA) in frog Ringer solution (in g l^{-1}: 0·84 $NaHCO_3$, 6·5 NaCl, 1·0 glucose, 0·16 KCl, 0·25 $MgSO_4.7H_2O$, plus 1·1 ml of 1 M $CaCl_2$.

Measurement of microvascular closing pressures

All tissues were transilluminated using a fibre-optic light source transmitted via a small quartz rod, and viewed through a Wild M10 stereomicroscope. Individual capillaries were cannulated with a sharpened glass micropipette (tip diameter, 12–16 μm), and perfused with a physiological saline solution containing 4·89 g (100 ml)$^{-1}$ Evans Blue (T1894, Sigma, with a dye content of 95%) and 5 g bovine serum albumin (100 ml)$^{-1}$ (BSA, Fraction IV, obtained from Sigma), such that more than 99% of the dye was bound to the albumin (Levick & Michel, 1973). The micropipette was attached to an adjustable water manometer and the zero level obtained by checking to see if fluid moved out of the micropipette tip when dipped into fluid on the surface of the tissue. The cannulation site was checked for leakage and if blue dye could be observed passing into the surrounding tissue or retrogradely in the vessel behind the pipette tip, the pipette was advanced until it fitted tightly within the vessel. Once the cannulation site was judged to be leak free, the pressure applied to the micropipette was adjusted until fluid movements between the micropipette and the lumen of the vessel were zero. This point was relatively easy to judge as a result of the coloured BSA–plasma interface. The pressure at this time equalled that at the first branch point downstream from the cannulation site where flow was occurring. This pressure was recorded as the intravascular pressure.

In order to measure the closing pressure (P_s) in the AVR, the manometer pressure was raised until the perfusate flowed freely through the vessel. The vessel was occluded downstream from the micropipette with a thin glass rod and the manometer pressure lowered from 15 cmH_2O over 15–30 s until the vessel collapsed. As soon as the vessel collapsed the pressure was noted and recorded as P_s; the procedure was repeated several times in each vessel. Once the collapse pressure was established, the pressure was lowered a further 1 cmH_2O, and then slowly raised until the vessel reopened. The pressure at which reopening occurred was recorded (P_o). The experiments were recorded on videotape using a Panasonic WV-BL200 CCD camera and a JVC Model CR-6600F recorder.

This general procedure for determining P_s in single AVR was repeated in rat DVR, rat spinotrapezius muscle venules, rat mesenteric capillaries and in frog mesenteric capillaries.

In addition to the above *in vivo* experiments, P_s measurements were performed on vasa recta *in vitro*. In the latter case the papilla was quickly removed from an anaesthetized rat and held in position via sutures under the dissecting microscope. The surface was kept moist with the modified mammalian Ringer solution (as above), individual vessels were cannulated and the closing pressure determined. Because the direction of flow could not be confirmed *in vitro*, ascending and descending vasa recta are grouped together in the results of these experiments.

Table 1. Closing pressures in individual microvessels

	Intravascular pressure (cmH_2O)	Closing pressure (cmH_2O)
Rat (*in vivo*)		
AVR	$5{\cdot}95 \pm 0{\cdot}15$ ($n = 83$)	$-3{\cdot}96 \pm 0{\cdot}13$ ($n = 57$)
DVR	$8{\cdot}71 \pm 0{\cdot}48$ ($n = 17$)	$-3{\cdot}94 \pm 0{\cdot}25$ ($n = 19$)
ST	$12{\cdot}84 \pm 0{\cdot}96$ ($n = 22$)	$-3{\cdot}21 \pm 0{\cdot}27$ ($n = 7$)
MES	n.d.	$-4{\cdot}24 \pm 0{\cdot}17$ ($n = 16$)
Rat (*in vitro*)		
AVR and DVR	n.a.	$-3{\cdot}04 \pm 0{\cdot}15$ ($n = 22$)
Frog (*in vivo*)		
MES	n.d.	$-5{\cdot}34 \pm 0{\cdot}22$ ($n = 16$)

All values are means ± S.E.M. n = number of vessels measured. Only one measurement was recorded per vessel, although this represented the mean of several trials. Each animal supplied a maximum of three vessels. n.a., not applicable; n.d., not done; ST, spinotrapezius muscle; MES, mesentery.

RESULTS

Transillumination, while perfusing rat ascending vasa recta (AVR) with Evans Blue albumin solution *in vivo*, allowed the edges of the vessel to be clearly visible despite a total thickness of the tissue of about 1 mm and the many layers of autoperfused capillaries underlying the vessel under study. When the pressure was lowered in the micropipette, the diameter of the lumen of the AVR could be seen to decrease slightly. As the pressure was lowered further over a period of a few seconds, however, the luminal contents paled until at a critical point the vessel appeared to snap shut, causing nearby vessels to suddenly shift position. The appearance of the vessel at this point was that of a fine blue line where residual dye adhered to the walls of the vessel. Since the vessel was occluded downstream, the fluid in the lumen must have moved back up into the micropipette.

Closing pressures in AVR *in vivo* were found to be negative, i.e. they were subatmospheric. The measurements were reproducible within individual vessels, since repeating the procedure while the micropipette was in place resulted in closure of the vessel at the same pressure. This procedure did not appear to damage the vessel since (a) there was no subsequent increase in albumin leakage through the walls as the manoeuvre was repeated, and (b) the results did not change with repetition. The pressure recorded was the mean of at least three trials on the same vessel.

Collapse of individual AVR and descending vasa recta (DVR) in the renal medulla of the rat occurred at a mean of approximately -4 cmH_2O (Table 1). P_s of the vasa recta *in vitro* behaved in a similar manner, with a mean of approximately -3 cmH_2O. There was a trend for the *in vitro* values to be slightly higher than those found *in vivo*, but the differences were not significant when assessed by Student's two sample t test (Wardlaw, 1985). Collapse of either the ascending or descending vasa recta could be seen to pull neighbouring vessels closer, suggesting some physical tethering to neighbouring vessels. Closing pressures of frog mesenteric capillaries (Table 1) were significantly lower ($P < 0{\cdot}05$) than those in the rat capillaries. Vessels in the mesentery are frequently separated from each other by large areas of avascular mesenteric tissue, and therefore no movement effects on nearby vessels were seen as the lumina snapped shut.

P_s was also measured in first order venules of rat spinotrapezius muscle (the first venule into which a true capillary was seen to empty). These vessels behaved in a similar manner to the vasa recta (Table 1), i.e. they did not shut until the luminal pressure was significantly below atmospheric.

An attempt was also made to quantify reopening pressures in two of the systems: frog mesenteric venules and rat AVR. This was done by observing the point at which the lumen became patent as the pressure was gradually raised. In the frog the mean P_s was $-5{\cdot}31 \pm 0{\cdot}49$ cmH_2O (mean ± S.E.M.; $n = 26$) and the mean reopening pressure (P_o) was $-4{\cdot}17 \pm 0{\cdot}38$ cmH_2O ($n = 17$). AVR did not immediately reopen when the intravascular pressure was raised above the collapse value, but required an additional 0·5–1·0 cmH_2O to reopen the vessel. The mean reopening pressure in the AVR was $-3{\cdot}41 \pm 0{\cdot}17$ cmH_2O compared with a mean closing pressure of $-4{\cdot}31 \pm 0{\cdot}17$ cmH_2O for the same group of sixteen vessels. After reopening, the vessel gradually refilled with pale blue fluid, the pale colour being possibly due to the dilution of dye remaining on the vessel walls by fluid reabsorbed from the interstitium while the lumen was under negative pressure.

DISCUSSION

In this study, closing pressures for individual capillaries in frog mesentery, ascending and descending vasa recta in the renal medulla and small venules in the spinotrapezius muscle of the rat were measured. The new finding is that individual microvessels are resistant to collapse, closing only at subatmospheric (negative) pressures. This was true for four different types of small vessel (three organs and two species) *in vivo* and also *in vitro* in the vasa recta of the isolated renal medulla. Our findings rest on the assumption that when a vessel collapsed the pressure within it equalled the pressure applied to the micropipette by the water manometer. There are good reasons for believing that this is at least approximately true. First, each micropipette was chosen to have a tip diameter comparable with the vessel diameter so as to minimize its flow resistance. Thus the very small flows resulting from fluid reabsorption into the occluded vessel can be driven into the micropipette by negligible differences in hydrostatic pressure. Second, the opening pressures in both vasa recta and frog mesenteric capillaries were subatmospheric and differed from the closing pressures by only about 1 cmH_2O. Since any small differences in pressure between the occluded vessel and the manometer should be of opposite sign during vessel opening and closing, the errors in our estimates of P_s must be less than 1 cmH_2O.

Thus, far from being inherently unstable at low intraluminal pressures, as suggested by Burton (1951) and Nichol *et al.* (1951), *individual* microvessels in the tissues studied here were able to resist collapse as intraluminal pressures were lowered well below atmospheric. One factor favouring stability of the microvessels may be the presence of structures for holding the vessel open, as suggested by Starling (1896). In the case of the renal medullary vasa recta, Takahashi-Iwanaga (1991) has demonstated by scanning electron microscopy how the interstitial cells ('sustentacular cells') have pseudopodia which completely encircle the vasa recta and adjacent loops of Henle, and partially encircle the collecting ducts, forming a horizontal ladder-like arrangement at 5–12 μm intervals, at right angles to the length of the vessels. The processes of the pseudopodia possess numerous terminal microvilli, which interdigitate with the microvilli of adjacent processes. The vasa recta in the inner one-third of the inner medulla all had endothelium with basal microvilli from 0·3 to 3 μm in length. In the outer two-thirds of the inner medulla only the venous (AVR) vasa recta had microvilli. Transmission electron microscopy showed that the microvilli connect with 'adjacent tubules or vessels by direct contact or by electron-dense matrices, [which] presumably serve to anchor the wall of the venous vasa recta to their surrounding structures, thus preventing collapse of the venous lumen' (Takahashi-Iwanaga, 1991). It is possible that less superficial vasa recta, in which micropuncture is not possible, may be even more strongly supported than those we investigated. At deeper sites, attachment of the basal microvilli will provide all-round support for the vessels rather than limited support from the sides or below. Since our estimates of P_s were made on relatively superficial vessels, they may underestimate the mean values of P_s for vessels of the inner medulla.

The observation that the lumina of the vessels did not close gradually but snapped shut, pulling neighbouring vessels with them, suggests a force which suddenly gives way at a critical point. Collapse of the lumen would require that both the integral stiffness of the vessel wall and any additional strength due to external physical tethering must be overcome.

In the case of the *in vitro* measurements on the vasa recta, both the initial intravascular pressure and the interstitial pressure should be close to atmospheric. The slightly higher closing pressures *in vitro* than *in vivo* suggests a small subatmospheric pressure in the interstitial space in contrast to the significant positive interstitial pressures which have been reported for the intact kidney *in vivo* (Garcia-Estañ & Roman, 1989). Since the physical tethering of the microvessels will remain the same, any difference between the *in vivo* and *in vitro* estimates of closing pressures might represent the contribution of interstitial pressure. It should be noted that *in vitro* as well as *in vivo*, the measured closing pressures may be at the higher end of the range (i.e. less negative) due to the fact that the vessels studied are near the exposed surface of the medulla, and only supported strongly on three sides.

The mean P_s for the frog capillaries (−5·34 cmH_2O) was significantly different from the mean of the three *in vivo* mammalian groups (−3·66 cmH_2O) at the 5% level. Our results for individual frog mesenteric capillaries are in contrast to those found for networks of frog mesenteric vessels by Nichol *et al.* (1951), where the diameters of individual vessels in a network became unstable at perfusion pressures of below about 20 cm saline. Flow ceased in some branches of the network at pressures of $+3{\cdot}3 \pm 1{\cdot}7$ cm saline (S.D.; $n = 29$) and in some cases reverse flow occurred. Nichol *et al.* (1951) assumed that cessation of flow indicated complete closure of the microvessels but they rarely observed this phenomenon directly. They reported the pressure at which flow stopped as the critical closing pressure, but a better description would be the term 'flow cessation pressure' (of Slaaf, Reneman & Wiederhielm, 1987). It seems likely that the flow cessation pressure is determined by factors such as the axial pressure gradient within the microvasculature and the rheological properties of the blood (Schmid-Schönbein, 1976). By contrast, microvascular closing pressures are determined by the transmural pressure differences and the mechanical properties of the

vessel walls. Thus flow cessation pressure may describe quite different properties of the microcirculation from the microvascular closing pressures reported here.

Our observations on the closing pressures of the vasa recta are potentially relevant to the uptake of fluid by the circulation in the renal medulla. *In vivo* the renal interstitium normally has a positive pressure of 5–10 cmH_2O (Garcia-Estañ & Roman, 1989), which may exceed the pressure in the AVR. Thus fluid may be driven from the interstitial space into the AVR by a small hydrostatic pressure gradient. Because the hydraulic permeabilities of the AVR are high, a small pressure difference (up to 4 cmH_2O) across the walls of the AVR, which does not collapse the vessel, could account for relatively large fluid movements, even in the absence of an oncotic pressure gradient.

In conclusion, we have demonstrated that individual vasa recta in the kidney can sustain a negative luminal pressure without collapse, a property which may facilitate reabsorption of interstitial fluid during urine concentration. In addition, this property appears to be common to other microvessels, e.g. those of muscle and mesentery, and may be quite separate from the phenomenon of flow cessation, which has been used in the past as an indicator of microvascular closure.

Aukland, K., Bogusky, R. T. & Renkin, E. M. (1994). Renal cortical interstitium and fluid absorption by peritubular capillaries. *American Journal of Physiology* **266**, F175–184.

Burton, A. C. (1951). On the physical equilibrium of small blood vessels. *American Journal of Physiology* **164**, 319–329.

Cuttino, J. T., Jennette, J. C., Clark, R. L. & Kwock, L. (1985). Renal medullary lymphatics: microradiographic, light and electron microscopic studies in pigs. *Lymphology* **18**, 24–30.

Garcia-Estañ, J. & Roman, R. J. (1989). Role of renal interstitial hydrostatic pressure in the pressure diuresis response. *American Journal of Physiology* **256**, 63–70.

Lassen, N. A., Longley, J. B. & Lilienfield, L. S. (1958). Concentration of albumin in the renal papilla. *Science* **128**, 720–721.

Levick, J. R. (1972). Studies on the permeability of individual capillaries. D Phil. Thesis, Oxford University.

Levick, J. R. & Michel, C. C. (1973). The permeability of individually perfused frog mesenteric capillaries to T1824 and T1824-albumin as evidence for a large pore system. *Quarterly Journal of Experimental Physiology* **58**, 67–85.

MacPhee, P. J. & Michel, C. C. (1992). Microvascular closing pressures of the vasa recta in the renal medulla of anaesthetized rat. *Journal of Physiology* **452**, 8*P*.

MacPhee, P. J. & Michel, C. C. (1993). Hydraulic permeability and osmotic reflection coefficient (σ) to bovine serum albumin in single perfused ascending vasa recta in the anesthetized rat. *International Journal of Microcirculation* **13**, 71.

MacPhee, P. J. & Michel, C. C. (1995). Fluid uptake from the renal medulla into the ascending vasa recta in anaesthetized rats. *Journal of Physiology* (in the Press).

Michel, C. C. (1984). Fluid movements through capillary walls. In *Handbook of Physiology*, section 2, vol. 4, *Microcirculation*, ed. Renkin, E. M. & Michel, C. C., pp. 375–409. American Physiological Society, Washington, DC, USA.

Nichol, J., Girling, F., Jerrand, W., Claxton, E. B. & Burton, A. C. (1951). Fundamental instability of the small blood vessels and critical closing pressures in the vascular beds. *American Journal of Physiology* **164**, 330–344.

Pallone, T. L. (1992). Molecular sieving of albumin by the ascending vasa recta wall. *Journal of Clinical Investigation* **90**, 30–34.

Schmid-Schönbein, H. (1976). Critical closing pressure or yield shear stress as the cause of disturbed peripheral circulation? *Acta Chirurgica Scandinavica Supplementum* **465**, 10–19.

Shrier, I., Hussain, S. N. A. & Magder, S. (1993). Effect of carotid sinus stimulation on resistance and critical closing pressure of the canine hindlimb. *American Journal of Physiology* **264**, H1560–1566.

Slaaf, D. W., Reneman, R. S. & Wiederhielm, G. A. (1987). Cessation and onset of muscle capillary flow at simultaneously reduced perfusion and transmural pressure. *International Journal of Microcirculation: Clinical and Experimental* **6**, 215–224.

Starling, E. H. (1896). On the absorption of fluids from connective tissue spaces. *Journal of Physiology* **19**, 312–326.

Takahashi-Iwanaga, H. (1991). The three-dimensional cyto-architecture of the interstitial tissue in the rat kidney. *Cell and Tissue Research* **264**, 269–281.

van Dijk, L. C., Krams, R., Sipkema, P. & Westerhof, N. (1988). Changes in coronary pressure–flow relation after transition from blood to Tyrode perfusion. *American Journal of Physiology* **255**, H476–482.

Wardlaw, A. C. (1985). *Practical Statistics for Experimental Biologists*, p. 49. John Wiley & Sons, Chichester, UK.

Acknowledgements

This work was supported by The Wellcome Trust (grant reference 187741/1.27) which also supported P. J. M. We would also like to thank Dr J. Marshall and her colleagues for demonstrating the spinotrapezius muscle preparation.

Received 17 January 1995; accepted 8 February 1995.