Pressure Measurements in Proximal Surface Tubules of the Rat—Single Nephron Filtration Rate and Tubuloglomerular Feedback

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Received for publication 19 November 1971

Recently much interest has centered upon the measurement of single nephron filtration rate (SNGFR) and on the evaluation of its regulation by means of an intrarenal feedback control mechanism. Methods used by many authors seem to differ technically, which may explain in part the conflicting results and the actual scatter of the data reported in the literature.

It has been pointed out by several groups that factors such as Na-intake(1), state of hydration(2), and sampling technique(3,4) affect nephron filtration rate. Furthermore, flow rate through the macula densa segment and flow rate dependent factors such as osmolality, Na-concentration, and Na-permeation have been considered regulating factors SNGFR in surface tubules(4–7). In many cases, however, the physiological flow of tubule fluid through the presumed receptor segment was interrupted at the time of SNGFR measurement. That interruption should affect the results obtained, if a tubuloglomerular control mechanism were in operation.

It is interesting to note that the highest values for SNGFR reported in the literature were obtained at interrupted distal flow(2,8–11). Exceptions are the data of Gertz et al.(3), who applied a special technique of sampling against the predetermined intratubular free flow pressure at stopped flow, and the data of Rouffignac et al.(12), who measured SNGFR at uninterrupted flow through the loop of Henle.

The most detailed information comes from a recent study by Schnermann et al.(13). During perfusion of loops of Henle with varying flow rates an inverse relationship between nephron filtration rate and perfusion rate was observed. It

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1 Supported by the Deutsche Forschungsgemeinschaft. Parts of the present paper were presented at the International Symposium on Renal Handling of Sodium, Brestenberg, August 2–5, 1971.

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was concluded that a correlate of the flow through the normal loop affects filtrate formation.

It is clear from these observations that measurement of SNGFR should ideally be performed under conditions of normal nephron perfusion.

A MEASUREMENT OF SNGFR

Recently, we have developed a technique(14) that appears to be suitable for the measurement of SNGFR according to the postulated criteria.

The recording device consists of a microperfusion pump (Hampel), a miniature pressure transducer (Kulite), and a single microcapillary. The system is shown schematically in Fig. 1. The three parts mentioned, i.e., glass capillary, pressure transducer, and microperfusion pump, are mounted in a Lucite block and connected by an oil filled, T-shaped central bore. The whole system, with the exception of only the tip of the capillary, is isolated by a PVC-mantle. Although the pressure transducer has been cemented into the Lucite pressure dome, the glass capillary is carried by a brass screw collar, which has a fine pitch thread at the outside, permitting careful introduction of the capillary into the central chamber without risk of damaging pressure spikes. Pressure equilibration during mounting is achieved through pressure escape slots cutting through the fine pitch thread.

The T-shaped pressure dome and part of the capillary are filled with silicone oil of 35 cSt viscosity and the rest of the capillary with indigo-carmine-stained saline or Ringer's solution to which drugs can be added.

![Diagram](attachment:fig1.png)

Fig. 1. Schematic diagram of microperfusion pump, pressure transducer, and microcapillary.
Figure 2 shows the calibration of the pressure recording system with different perfusion rates using a high resistance (10 μm o.d., long tip), and a low resistance (12 μm o.d., short tip) capillary. The vertical lines indicate maximal pressure fluctuations which are due to ripple of the pump. Sufficient linearity was observed under these conditions as well as when external pressure was applied to the tip. At the optimal supply voltage, which has been tested after mounting the transducer, stability of zero pressure was observed for more than 2 hr.

In Fig. 3 a typical experiment is depicted schematically. First the effective tip resistance, w, of the perfusion capillary was tested with the capillary tip in aqueous phase on the kidney surface. Subsequently the tip penetrated into the lumen of a proximal convolution and intratubular pressure (P_{tub}) was recorded at zero perfusion. The pressure increased immediately upon starting the intratubular perfusion at a rate of 5 nl/min as indicated on the bottom. As shown on top, a second capillary filled with Sudan black-stained castor oil was
then introduced into the same loop as far proximal as possible and an oil column
was injected into the tubular lumen, which extended proximally and distally
from the injecting capillary tip. As soon as the oil droplet had drifted into the
position shown in Fig. 3 (i.e., into the nephron segment between the two tips) a
brief aspiration, if any, initiated collection of TF that continued spontaneously
in most of the experiments.

Simultaneously, the pressure in the downstream segment decreased and ap-
proached at a value equivalent to a perfusion rate of 5 nliters/min. Flow rate
was then elevated stepwise until intratubular pressure exceeded that prior to oil
blockade or perfusion rate was adjusted to equal that of the free flow state. Un-
der these circumstances $P_{\text{tub}}$, corrected for the effective tip resistance, equaled that
of the initial measurement. Under ideal situations pressure-controlled flow rate
and rate of spontaneous collection remained stable for a sampling period of
5 min, allowing enough TF in the collecting capillary for quantitative determina-
tion of volume and $^{14}$C-inulin concentration.

Thus, fluid collection in the upper segment was performed under conditions
of pressure-controlled normal perfusion of Henle’s loop. A suprablockade SNGFR
could be calculated according to

$$
\text{SNGFR}_{\text{(supra)}} = \hat{V}_{\text{(TF)}} \cdot \text{inulin} \ (\text{TF/P}).
$$

Since the distance of the two probing capillaries was in the order of 200 µm,
i.e., below 5% of the total length of the proximal convolution, a second estimate
of SNGFR could be obtained from

$$
\text{SNGFR}_{\text{(sub)}} = \Phi_{\text{(at } P_{\text{tub}})} \cdot \text{inulin} \ (\text{TF/P}).
$$

The experiments which will now be demonstrated have been carried out with
male Wistar rats of ca. 200 gm body wt, anesthetized with inactin (80 mg/kg
body wt). The animals were antidiuretic with inulin U/P ratios ranging from
350 to 900. The perfusate consisted of isotonic saline stained with indigo-carmine
in order to estimate semiquantitatively the position of the puncture site.

In Fig. 4 23 double measurements of SNGFR have been plotted. They appear
to scatter randomly around the dotted line of identity. From the data available
so far, SNGFR as calculated from pressure-controlled measurements ranges in the
order of magnitude of 21 nliters/min, which is equivalent to 90 nliters/min • kg
body wt. We consider these values to be preliminary until a repetition of the
measurements using TF as perfusate has confirmed the results.

The present values are lower than many reported in the literature. Therefore
consideration must be given to the possibility that leakage of TF occurred along
the brush border oil interphase from above to below the oil blockade. This
would decrease the volume of collected TF, as well as the rate of perfusion re-
quired for pressure restoration. We feel that this error was not a factor in our
measurement because under optimal sampling conditions the oil block remained
in a stable position and different lengths of the oil blockade did not reveal ap-
parent differences in results. Finally, the pressure flow characteristic of the down-
stream segment was not influenced, even when up to 10 loops of the upstream
segment were blocked by mineral oil, a situation in which contamination by leakage can safely be excluded.

B PROXIMAL INTRATUBULAR Pressures AT DIFFERENT FLOW RATES THROUGH HENLE’S LOOP

An obvious application of the present setup is the continuous recording of proximal intratubular pressures at different flow rates through the loop of Henle. If proximal TF is collected at a constant rate a tubuloglomerular signal from a more distal site that would reduce effective filtration pressure, should result in a decrease of the pressure measured by the collecting capillary. Elevation of effective filtration pressure on the other hand should be reflected by an increase of intratubular pressure.

The second protocol (as shown in Fig. 5a) was as follows.

A capillary, carrying a pressure-recording microperfusion system, was introduced into an early proximal convolution for the measurement of the intratubular pressure under normal free flow conditions.

By infusing an indigo–carmine-stained Ringer’s solution the downstream loops of the same nephron were identified. A second similarly equipped microcapillary (shown in Fig. 5b) was inserted into the last accessible loop. Perfusion through capillary no. 1 was stopped and intratubular pressure was simultaneously recorded at both puncture sites. Then, three maneuvers (shown in Fig. 5c) were carried out in rapid sequence: The loop of Henle was perfused via capillary No. 2 with 5 nliters/min, while collection of tubular fluid was started through capillary No. 1, using the perfusion device as a constant volume suction pump. Immediately afterward, an oil column was injected with a third capillary into
the intermediate segment, to block linear flow of tubular fluid. The rate of fluid collection by pump No. 1 was then adjusted until the oil column remained stable in position, and the perfusion rate through capillary No. 2 was elevated until the normal intratubular pressure ensued. Thus, the tubule under investigation was functionally separated by the oil blockade into two segments: an upper segment, which drained quantitatively into the collecting capillary, and a lower segment, which was externally perfused via the pump system at the physiological flow rate.

In the experiments to be presented flow rate was altered as soon as the system had stabilized, by either increasing or decreasing the rate of the perfusion pump in the range from 0 to 35 nliters/min. The animals were antidiuretic, male Wistar rats, 180–270 gm body wt. They were anesthetized with pentobarbital (prime dose 65 mg/kg body wt, sustaining perfusion 30 mg/kg body wt · hr).

Figure 6 shows the continuous recording of an experiment in which a feedback signal was observed. A stable situation was reached at a suction rate of (−) 12 nliters/min and a perfusion rate of (+) 7 nliters/min. Decreasing the rate of per-
Pressure record from individual experiment in which perfusion rate through the loop of Henle was changed. Note proximal tubular pressure fall with increase in loop perfusion rate.

Perfusion had no clear-cut effect, but increasing it to 15 n liters/min resulted in a rapid decrease of intratubular pressure with a visible tubular collapse. Restoration of the intratubular pressure was observed, when perfusion rate was subsequently lowered again. As is shown in Fig. 6 the observation could be repeated in the same tubule.

The existence of a tubuloglomerular signal could not be demonstrated in all tubules of the same kidney as shown by Fig. 7. Here, proximal intratubular pressure remained unchanged despite changes of the rate of perfusion from 0 to 35 n liters/min.

In the present experiments we have observed

(a) no effect of either increasing or decreasing perfusion rate out of the proximal convolution in nine tubules of five animals.

(b) A clear-cut tubular collapse within 30 sec was observed in 16 tubules of nine animals when proximal tubular outflow was elevated above the normal range and proximal collection rate was constant. In all cases tubular collapse could be reversed by decreasing or interrupting perfusion rate.

(c) Lowering proximal tubular perfusion below the physiological range had no clear-cut effect on intratubular pressure above the oil blockade, when the collection rate was kept constant. However, up to the present we have not extended our observation beyond 3 min.

The results seem to differ from those reported by Morgan(15) and are to some extent consistent with the observation of Schnerrmann et al.(13). The reason for
the difference in the reported results is not clear from the techniques used and from the protocols published.

With regard to pressure changes, it is interesting that in our experiments we have obtained both positive and negative results. Thus the data differ from nephron to nephron and not from rat to rat. The status of the animal during the experiment is apparently not the reason for the differing responses of individual nephrons. We interpret our present results as follows.

(a) In a great number of surface tubules, changes in outflow out of the proximal convolution can affect intratubular pressure in the proximal convolution and apparently also SNGFR by a signal originating in the same nephron below the oil blockade. However, we cannot deduce from our experiments that Na-reabsorption at the macula densa segment and Na-dependent angiotensin activation are in the chain of events.

(b) The relative ineffectiveness of lowering the perfusion rate through the loop of Henle could be caused by a reversal of intratubular flow such as has been observed by Brandis et al.(16), and Steinhagen et al.(17).

(c) Regardless of the mechanism of action of the tubuloglomerular feedback, it cannot be demonstrated in all surface tubules. This could either be due to a technical insufficiency, or, more likely, to a biological scatter among
nephrons in such a way, that in a number of surface tubules the signal fails to reach that individual nephron.

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