Kidney International, Vol. 24 (1983), pp. 800–803

Dynamic evaluation of renal electrolyte gradient by in situ tissue impedance studies

JANUSZ SADOWSKI and EWA PORTALSKA
with the technical assistance of JADWIGA ZWOLIŃSKA

Department of Applied Physiology, Medical Research Center of the Polish Academy of Sciences, Warsaw, Poland

The combined operations of the loops of Henle (countercurrent multiplier) and the vasa recta (countercurrent exchanger) establish hypertonicity of the renal medulla with respect to the cortex. The extent of this hypertonicity or the magnitude of the cortico-papillary solute concentration gradient is of obvious physiological interest: The hypertonic milieu provides a driving force for water reabsorption in the collecting system and reflects the rate of solute delivery from the tubules to the interstitium as opposed to solute "washout" from the interstitium by the vasa recta blood.

Since electrical resistance of an electrolyte solution is inversely related to the concentration of free ions, one may expect that electrical impedance of a tissue would be a function of electrolyte concentration in tissue fluid. With this assumption in mind we developed a method for tissue impedance measurement by means of needle electrodes and applied this method for physiological studies of the cortico-papillary electrolyte gradient of the in situ dog and rabbit kidney. The major advantage of this approach is that, unlike usual tissue analytical techniques, it enables a dynamic evaluation of the electrolyte gradient (though not urea or total osmole gradient) in a living and functioning organ, and it makes possible the analyzation of the tissue electrolyte gradient responses to various stimuli.

Methods. When an alternating current is applied to biological tissue, tissue impedance can be defined as $Z = \sqrt{R^2 + X_c^2}$, where R denotes resistance and X_c is capacitive reactance. Unfortunately, measuring electrodes' own impedance due to their polarization is to be considered, and then the apparent impedance measured is a sum of tissue impedance and electrode polarization impedance. The contribution of the latter to the overall value decreases with the increasing active surface of electrodes (that is, decreasing current density) and with the increasing AC frequency used, particularly within the range up to 10^4 Hz [1].

Electrodes for kidney tissue impedance measurement were prepared from 75% platinum/25% iridium wire, 0.5 mm in diameter; the whole except 1.2 mm length of a sharpened tip was insulated with a polyurethan varnish. Pairs of electrodes (one pair forming an impedance cell) were fixed at the base in a cuboidal bedplate made of metacrylate resin so that the distance between two electrodes in a pair remained constant. For cells used on dog kidney this distance was about 2.1 mm and for rabbit cells 1.7 mm. An electrode set used in the rabbit comprised two pairs of electrodes and three pairs of electrodes fixed in one bedplate for the dog. Two rabbit sets (two pairs in each) comprised electrodes that were 2, 6, 9 and 15 mm in length (Fig. 1). In two dog sets (three pairs in each) electrode length was 4, 8, 12, 16, 20, and 23 mm.

The studies of tissue impedance presented here require no special equipment: Any of the typical commercial devices commonly used for measurement of the conductance of electrolyte solutions can be utilized. We used a laboratory conductometer (N-572, Mera-Elwro, Poland) which provided an input voltage generation of a 400 or 3500 Hz frequency. When the device is applied for solutions, owing to the design of the circuitry and the standard measuring cell, the contribution of the capacitive reactance (X_c) is virtually eliminated, and the conductometer automatically transforms the output voltage into a reciprocal of resistance (conductance). When, instead of the standard conductance cell, our needle electrodes for tissue studies were used, the X_c component could not be neglected and the readings represented a reciprocal of impedance, that is, admittance ($Y = Z^{-1}$), expressed, as before, in siemens (S). Since conductance (and not resistance) is a linear function of free ion concentration of a solution, we may also expect that admittance rather than impedance would be more directly related to tissue electrolyte content.

The conductometer provided an automatic compensation for tissue temperature changes: Rectal temperature signal (Fig. 1) was fed to the U/I convertor in the output portion of the measuring circuit. The value of the temperature coefficient selected for this compensation was 2%/°C, as recommended for biological tissue [2]. Later experiments in which a thermocouple was inserted into the kidney showed that such temperature compensation was not very critical as tissue temperature changes during a study rarely exceeded 0.5°C.

Admittance values were continuously recorded using a one-channel potentiometric line recorder.

Rabbits and dogs were anesthetized with intravenous sodium pentobarbital, 25 mg/kg body weight. The left kidney was exposed from a flank incision. In the dog the kidney was placed in a plastic holder positioned in a way to minimize stretching of the renal pedicle. In the rabbit which shows less vigorous

Received for publication January 18, 1983
and in revised form May 13, 1983

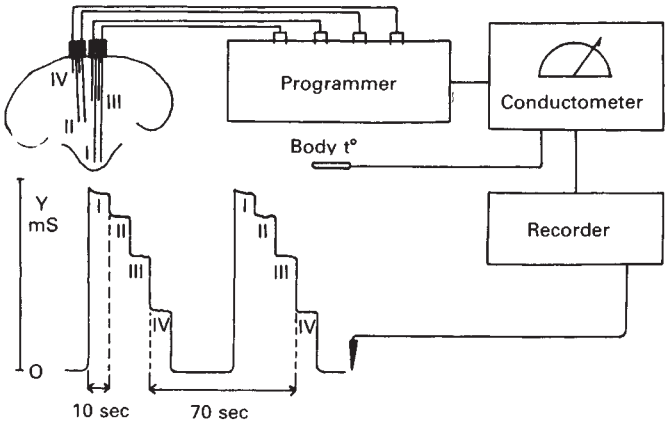

Fig. 1. *An experimental set-up for sequential recording of tissue admittance* (Y) *in four different zones of rabbit kidney.* Four electrode pairs are turned on and off in sequence by a programming device. The cycle is repeated every 70 sec, after 10 sec of activity of each electrode pair, 60 sec pass before it is activated again. The tip-to-base length values for individual electrode pairs (assembled in two sets: I/III and II/IV) were: I—papilla, 15 mm; II—inner medulla, 9 mm; III—outer medulla, 6 mm; IV—cortex, 2 mm.

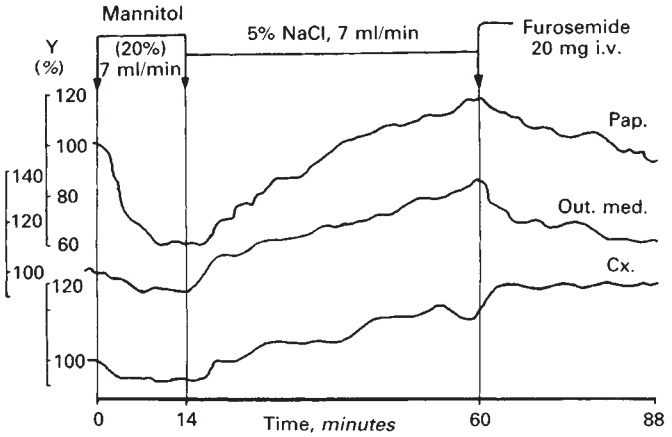

Fig. 2. *Admittance* (Y, 400 Hz) *responses to hypertonic mannitol infusion followed by hypertonic sodium chloride and then by an injection of furosemide (dog kidney).* Percent changes from control are shown.

respiratory movements that do not disturb admittance recording, the kidney simply rested on the animal's flank. When all other preparations required for urine collection, blood pressure, and renal blood flow measurement were completed, two sets of electrodes (each comprising two electrode pairs in the rabbit and three pairs in the dog) were inserted into the kidney. It was attempted to direct the longest pair of electrodes in the longitudinal plane from kidney surface toward the tip of the papilla (Fig. 1) and with some experience this was almost always successful. The recording of admittance during the process of impalement was helpful in detecting misplacement: When one or both electrodes of the longest pair pierced the tissue and entered the lumen of the calyx, the admittance measured showed unusual oscillations. Possibly, the electrode's active surface was then making contact alternately with the urine, the calyx wall or with both, in different proportions. In such cases a slight withdrawal restored a steady record pattern. Insertion of the electrodes did not reduce or produced only a transient reduction of the renal blood flow measured by a Doppler probe on the renal artery.

The experimental set-up for kidney admittance studies is schematically shown in Figure 1. The electrodes reaching different kidney zones from cortex to papilla are turned on and off in sequence by a programmer, and the whole cycle is repeated every 70 sec. As the measuring process itself could affect electrolytes of the functioning kidney tissue and the admittance was observed to drift slightly during measurement, interrupted rather than continuous recording was used. This provided a 60-sec resting period following each 10 sec of activity of any electrode pair.

Results and Discussion. An example of tissue admittance (Y) responses (measured at 400 Hz) to experimental maneuvers which would affect kidney tissue electrolyte content is shown in Figure 2. Hypertonic mannitol infusion produced a decrease in Y, particularly in the papillary region, probably reflecting a well-known phenomenon of "washout" of medullary electro-

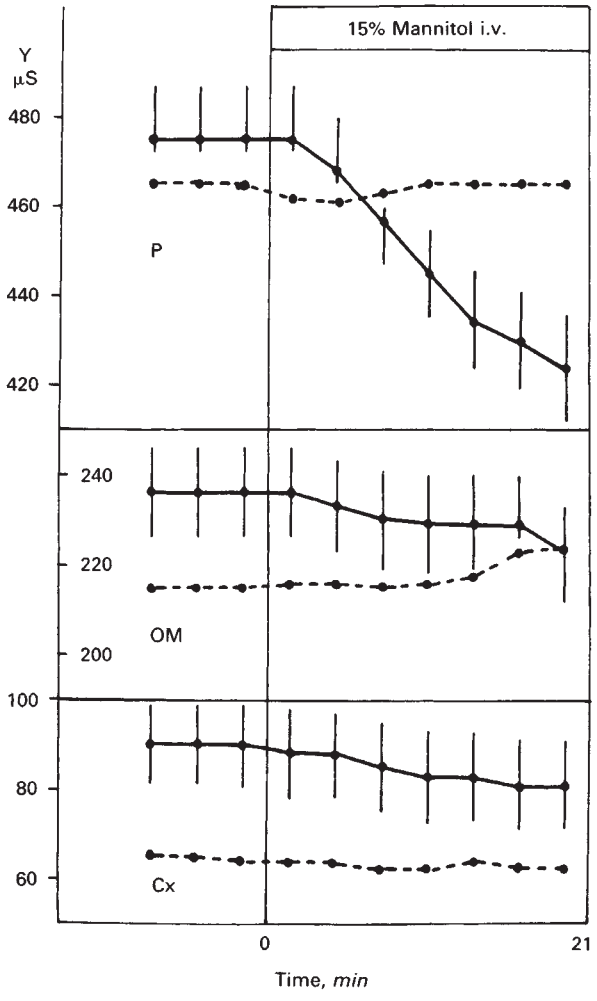

Fig. 3. *Admittance* (Y, 400 Hz) *responses of the papillary* (P), *outer medullary* (OM) *and cortical* (Cx) *tissue to intravenous mannitol infusion in seven anesthetized rabbits* (means ± SEM). Mean values for three noninfused animals are shown for comparison.

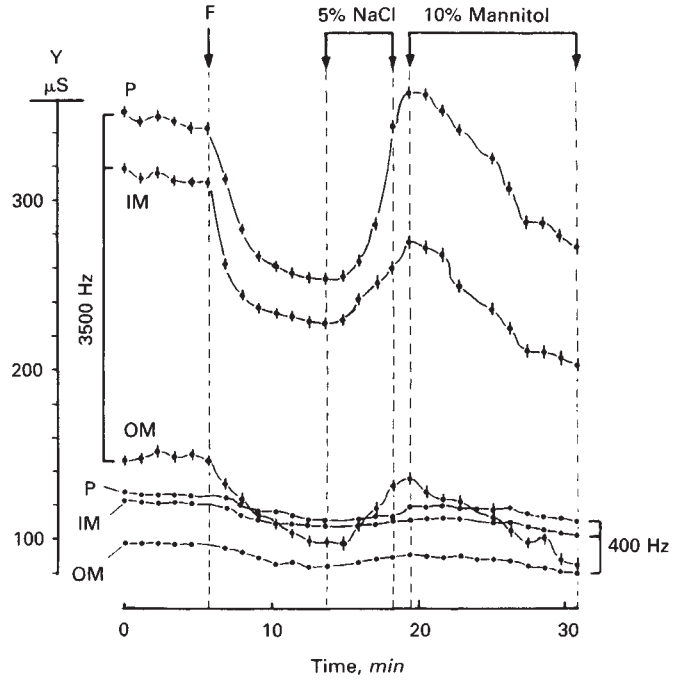

Fig. 4. *A comparison of 400 Hz- and 3500 Hz admittances* (Y) *as affected by small doses of furosemide* (F), *hypertonic saline and mannitol in single experiment with a dog kidney.* Abbreviations are: P, papilla; IM, OM, inner and outer medulla.

lytes during osmotic diuresis. An increase in Y measured during subsequent hypertonic sodium chloride infusion was conceivably related to increased sodium chloride delivery to and reabsorption from the renal tubules (increased delivery to the interstitium) whereas an opposite effect was observed with subsequent blocking of sodium chloride reabsorption with furosemide. Such responses were reproducible from experiment to experiment: Figure 3 shows admittance changes (also measured at 400 Hz) during mannitol infusion observed in a group of seven anesthetized rabbits. Again, the most pronounced decrease in Y was measured by electrodes located in the papillary region. However, it will be shown below that admittance values of different kidney zones cannot be directly compared.

We suspected that at 400 Hz the electrode polarization impedance constitutes a large constant fraction of the total impedance measured; reducing this contribution might render the method more "sensitive" to change in tissue electrolyte concentration. This could be accomplished by increasing the active surface of the electrodes. However, platinization of the tips to produce platinum-black surface would mean a loss of reproducibility of the results, as platinum particles would partly detach during each electrode impalement [2]. Therefore, we preferred to test if the presumably high polarization impedance could not be reduced by increasing the frequency to 3500 Hz. Figure 4 shows that, indeed, using this frequency dramatically amplified tissue admittance changes which followed furosemide, mannitol, or hypertonic saline, all three agents administered at doses distinctly smaller than those used previously to demonstrate admittance changes measured at 400 Hz.

To evaluate the relation between tissue Y measured at 400 or

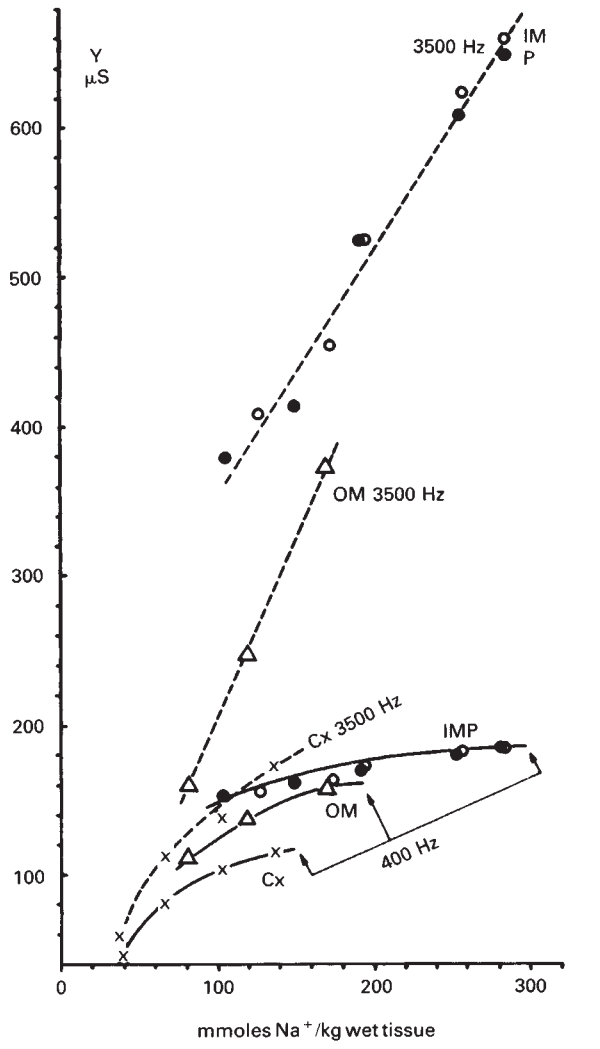

Fig. 5. *Tissue calibration data for the dog kidney.* Admittances of 400 Hz- and 3500 Hz of the papillary (P, *closed circles*), inner medullary (IM, *open circles*), outer medullary (OM) and cortical (Cx) tissue are related to tissue Na^+ content.

3500 Hz and the electrolyte content, tissue calibration studies were performed. Pre-weighed slices (about 250 mg) of dog kidney papilla, inner medulla, outer medulla, and cortex were placed in erlenmayer flasks containing 40, 100, 160, 220, 280 and 340 mM saline. After shaking the samples at room temperature during 90 min, their admittance was measured at 400 and 3500 Hz, and their actual Na^+ content was determined by extraction with boiling distilled water as described by Appleboom et al [3]. The results of these studies are summarized in Figure 5 which shows that: *(1)* The admittance measured was not only related to tissue Na^+ but critically depended on the zone of the kidney. While the relation of Y to $[Na^+]$ for the papillary and outer portion of the inner medulla was visibly described by one curve, the curves for the outer medulla and cortex were quite different. *(2)* At the same Na^+ concentration the admittance measured were distinctly higher at 3500 than at 400 Hz. *(3)* For the ranges of tissue Na^+ concentration that are of physiological interest here, the Y to $[Na^+]$ relation was linear

or close to linear when Y was measured at 3500 Hz; at 400 Hz the relation was curvilinear and the slope of the curves was reduced markedly. This explains higher "sensitivity" of the method at 3500 Hz (Fig. 4).

When measured at low frequencies, the impedance of cell membranes is one to three orders of magnitude higher than that of the extracellular fluid [2]. This means that the current bypasses cells, flowing almost exclusively through the extracellular fluid. Therefore, the admittances measured here were assumed to be primarily those of the extracellular compartment. To substantiate this assumption we measured 400 and 3500 Hz admittances of blood with hematocrit values ranging from 0 (no cells in the medium) to 100% (no intercellular fluid). The packed cells' admittance (Ht 100%) measured with the same electrodes and sensitivity setting of the conductometer as those used with tissue studies equalled zero for both frequencies. Apparently, impedance of the cells was so high that their admittance was not measurable. Pure plasma admittance at 400 Hz was one fifth of that measured at 3500 Hz, presumably because of high electrode polarization impedance at the lower frequency.

In summary, we believe that with appropriate precautions and tissue calibration tests, measurement of the electrical admittance of the renal tissue provides a useful tool for dynamic evaluation of the cortico-papillary electrolyte gradient. The fact that admittance seems to be an index of mostly extracellular electrolyte concentration is an advantage as any information on changes in this compartment of the renal medulla would be of crucial physiological importance. It must be considered that the withdrawal of water from the medullary collecting ducts (that is, urine concentration) depends on effective solute concentration in the extracellular fluid. Moreover, any modification of the tubular electrolyte transport would affect the surrounding extracellular milieu primarily.

Acknowledgments

This work was supported by Poland's National Research Program 10.4.03. The authors thank Drs. R. Grucza and R. Gellert for cooperation in the initial studies using the method described in this work.

Reprint requests to Dr. J. Sadowski, Department of Applied Physiology, Medical Research Center of the Polish Academy of Sciences, Jazgarzewska 17, 00-730 Warsaw, Poland

References

1. GEDDES LA: *Electrodes and the Measurement of Bioelectrical Events*. New York, Wiley-Interscience, 1972, pp. 14–25
2. SCHWAN HP: Determination of biological impedances, chap 6, in *Physical Techniques in Biological Research*, edited by NASTUK WL, New York and London, Academic Press, 1963, vol 6, pp. 323–342
3. APPELBOOM JWT, BRODSKY WA, TUTTLE S, DIAMOND I: The freezing point depression of mammalian tissues after sudden heating in boiling distilled water. *J Gen Physiol* 41:1153–1169, 1958