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In Vivo Study of Transepithelial Potential Difference (TEPD) in Proximal Convoluted Tubules of Rat Kidney by Synchronization Modulation Electric Field

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Abstract Synchronization modulation (SM) electric field has been shown to effectively activate function of Na^+/K^+ pumps in various cells and tissues, including skeletal muscle cells, cardiomyocyte, monolayer of cultured cell line, and peripheral blood vessels. We are now reporting the in vivo studies in application of the SM electric field to kidney of living rats. The field-induced changes in the transepithelial potential difference (TEPD) or the lumen potential from the proximal convoluted tubules were monitored. The results showed that a short time (20 s) application of the SM electric field can significantly increase the magnitude of TEPD from 1–2 mV to about 20 mV. The TEPD is an active potential representing the transport current of the Na/K pumps in epithelial wall of renal tubules. This study showed that SM electric field can increase TEPD by activation of the pump molecules. Considering renal tubules, many active transporters are driven by the Na^+ concentration gradient built by the Na^+/K^+ pumps, activation of the pump functions and increase in the magnitude of TEPD imply that the SM electric field may improve reabsorption functions of the renal tubules.

Keywords Transepithelium potential difference · Active potential difference · Proximal convoluted renal tubule · Synchronization modulation

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Introduction

Kidney is a vital regulatory organ in our body. Function of kidney is to accurately maintain the volume, composition, osmolarity and, ionic concentrations in blood. The functional unit of kidney is numerous nephrons each of which consists of glomerulus covered by a hollow Bowman's capsule followed by a renal tubule. Blood is filtered at the glomerulus but many filtrated biomaterials, such as amino acids, proteins, glucose, most of Na^+, K^+ ions, phosphate, and bicarbonate, are reabsorbed back into capillary blood vessels by various active transporters in the renal tubular epithelial wall. The metabolic waste products including uric acid, urea, and creatinine are excreted into the tubules which flow with extra water to bladder through ureters and eventually become urine excreted from body.

Na^+/K^+ pumps play a key role in the reabsorption functions of kidney. All the segments of the renal tubule including proximal tubule, loop of Henle, distal tubule, and collection tubules have high density of the pump molecules. Kidney is the organ having the richest Na^+/K^+ pumps in our body (Terada and Knepper 1989). A close relationship exists between the density of pump molecule and the reabsorption capacity in all segments of the renal tubules (Burnett et al. 1982; Bartoli et al. 1996). In each pumping cycle, the pump molecule extrudes 3 Na^+ ions form cell and pumps in 2 K^+ ions by consuming energy form one ATP hydrolysis to maintain the ionic concentration gradients. The pump molecules mainly located on the basal lateral membrane of the epithelial cells extrude Na^+ ions to the peritubular space, and therefore, build up a high Na^+ concentration gradient across the apical membrane of the cell. The electrochemical energy stored in the Na^+ concentration gradients is the driving force for many co-transporters (Harris et al. 1986; Bomsztyk 1986; Romero et al. 2004; Alexander and Grinstein 2006) on the apical membrane to

actively transport the filtrated biomaterials from lumen to the peritubular space, eventually getting into capillary blood vessels (Feraille and Doucet 2001). Functions of Na^+/K^+ pumps are directly related to the reabsorption functions of the renal tubules and, therefore, kidney functions.

Many kidney diseases involve dysfunctions of the pump molecules due to either insufficient ATP supplies, deficit in the regulation system, or reduction in the abundance of the pump molecules. Maintenance or restoration of the pump functions in renal tubule is a critical step towards therapeutic treatment of the kidney diseases.

Practically, it is not easy to activate any active transporter, such as Na^+/K^+ pumps. Even though the pumps are sensitive to the membrane potential, it is difficult to effectively activate the pump functions by an electric field because the Na^+ and K^+ ions are transported to the opposite directions and, therefore, having reversal voltage-dependence. Any membrane potential change can only facilitate one transport but inevitably hinders the other. In addition, for intact cells an external electric field will have opposite effects on the two cell hemispheres, one is depolarized while another hyperpolarized. If the pump molecules in one hemisphere are activated, the pumps in another hemisphere will be deactivated.

Recently, we developed a new technique, so called synchronization modulation (SM) which can effectively activate the pump functions by a well-designed oscillating electric field. The underlying mechanisms involved the SM have been reported previously (Chen 2008). Briefly, based on the sequential pattern of the two transports in the pumping cycle and their opposite voltage-dependence, we can apply a specially designed oscillating electric field to the cell membrane so that the field in two half-cycle can alternatively facilitate the two transports, respectively. However, due to random pumping phase and different pumping rate of individual pumps, it is impossible to use one electric field to fit all the pumps. Therefore, the first step we have to do is to synchronize the pump molecules. To do so, the oscillating electric field is designed based on the physiological parameters of the pump molecule so as to the Na^+-transport can only occur during the period of the field positive half-cycle and K^+-transport in the negative half-cycle, which is achieved by designing the two half-cycles as two energy-traps for the two transports, respectively. As a result, all the pumps are synchronized by the electric field running at the same pumping pace as the field oscillating frequency. Then, by carefully maintaining the pump synchronization and gradually changing the synchronization frequency, either increase or decrease, we can progressively and effectively entrain the pump molecules to run at a predefined pumping rate.

The whole-cell patch clamp experiments on skeletal muscle fibers in direct measurement of the pump currents have shown that the pumping rate can be effectively synchronized and accelerated up to ten-fold rapidly in about 10 s (Chen et al. 2007, 2008). The technique has been used in several labs on various cells and tissues, including intact skeletal muscle fibers (Chen and Dando 2006, 2007), slice of cardiomyocytes (Dando et al. 2012), peripheral blood vessels segment (Zhang et al. 2012), and cultured monolayer of MDCK kidney cells (Tran et al. 2013). The results showed that the SM electric field can effectively activate the pump functions, hyperpolarizing the membrane potential, and increasing the transepithelial potential difference. In study of the MDCK cells, Chao and his associates (Tran et al. 2013) at UC Davis found that the SM electric field can effectively increase the transepithelial potential difference across monolayer of the cultured MDCK cells, and confirmed with various methods that the TEPD increase is due to activation of the Na^+/K^+ pumps.

Based on these studies, our hypothesis is that the SM electric field, when properly applied to kidney of living animal, should be able to activate functions of the Na^+/K^+ pumps in the epithelial cells of renal tubules, and therefore, increase the TEPD. Consequently, the SM electric field may be able to improve reabsorption function of renal tubules, and therefore, the kidney functions. Recently, we conducted a series of in vivo studies of living animals by application of the SM electric field to kidney of rat. Here, we report the results in monitoring the field-induced increase in the magnitude of transepithelium potential difference from proximal convoluted tubules (PCT) of rat kidney.

The results showed that the SM electric field can significantly increase the magnitude of transepithelium potential difference in PCT for up to ten-time quickly in tens of seconds. The results imply that the SM electric field by activation of the Na^+/K^+ pumps in the epithelial wall may be able to improve tubular reabsorption functions, and therefore, the kidney functions.

Methodology in Measurement of Transepithelium Potential Difference, SM Electric Field, and the Field Application

Measurement of the transepithelium potential difference Rat was anesthetized by intraperitonealy injection of Inactin (100 mg/kg), and then placed on a temperature-controlled platform with a heat pat adjusted to 37–38 °C. Upon anesthesia, a flank incision was made to expose kidneys, and the kidney was held by a special ring holder. To maintain moisture, the kidney surface was coated by PBS solution periodically. Under a Leitz stereo microscope with long working distance the renal tubules, mainly the segment of PCT, can be identified on the surface of kidney. A pipette microelectrode with a tip of about 1-μm orifice filled with 3 M KCL was adjusted by an electrically

controlled manipulator to be punctured into the lumen of the PCT segment along the axis of the segment. The electrode was connected, through a head stage, to a Dagan TEV-200 in record mode to measure the transepithelium potential difference. A sharp pin reference electrode was slightly impaled into the kidney surface. The measured potential difference was stored in a PC using an analog to digital converter BNC 2110 (National Instruments) for later analysis.

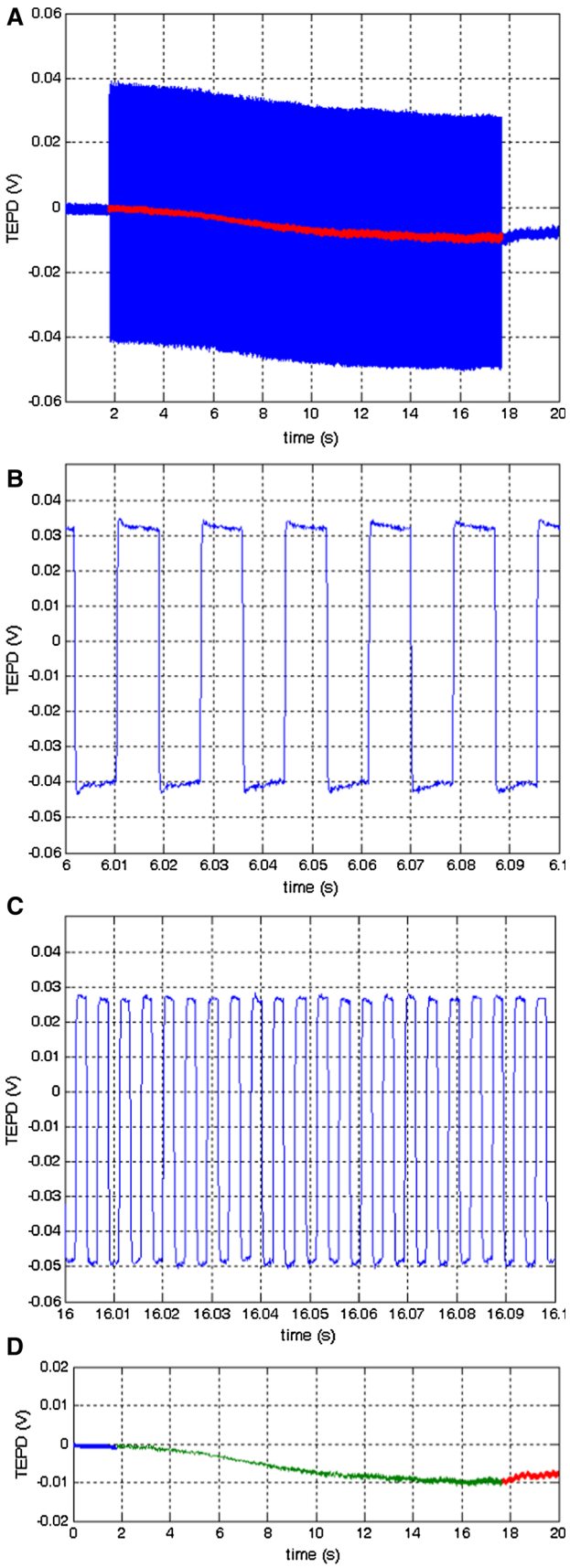
The synchronization modulation electric field was generated from a custom-made device. There are three modulation modes: forward- and backward-modulation, as well as no-modulation (constant-frequency). The first two modes consist of two stages, synchronization and modulation. Both stages have the same pulsed waveform and the magnitude except the oscillating frequency. The synchronization stage consists of 100 oscillating pulses of 50 Hz. This frequency is comparable to the natural turnover rate of the Na^+/K^+ pumps (Rakowski et al. 1989). In the modulation stage, the field frequency is gradually increased in the forward-modulation mode, or decreased in the backward-modulation mode in a stepwise pattern. The frequency step was 3 % for every 20 pulses until reaching the final frequency of 500 Hz (forward-modulation), or 10 Hz (backward-modulation). For the no-modulation mode, the field frequency remains the same throughout the field application. The detail frequency is identified in each experiment.

Application of the oscillating electric field The electric field is applied to the kidney by a pair of stainless steel pin-electrodes with sharp tip. The electrodes were placed on the two hemispheres of the kidney across the site of the two measurement microelectrodes. The pin electrodes were either slightly impaled into the surface of kidney or placed contacting the kidney surface without impalement. The former may cause a little, transient bleeding, while the disadvantage of the latter is a possible movement of the electrodes during the measurement. The results obtained from two methods did not show significant difference.

The potential difference applied to the two electrodes is in a range from a few hundreds mV up to 1 V depending on the position of the field-application electrodes and orientation of the PCT segment we measured. The field-induced oscillating TEPD in the proximal tubule was monitored by the microelectrode. For each experiment, the strength of the applied electric field was adjusted so that the field-induced oscillating TEPD was in the physiological range from 40 to 50 mV.

Experimental Results and Data Analysis

Figure 1 shows the lumen potential or the transepithelium potential difference measured through the microelectrode



◀**Fig. 1** **a** Transepithelum potential difference measured from PCT in kidney of rats when the kidney is exposed to the SM electric field. The field is applied at about 1.7th second for 16 s. Before the field application, the potential difference is about −1 mV with lumen negative. The field-induced potential oscillation is about 40 mV. Within the first 2 s (synchronization stage) the profile of the field-induced oscillation is symmetric with respective to the resting TEPD of −1 mV. Then, the oscillation profile is gradually bent to the negative direction in the modulation stage. Right after removal of the electric field, the TEPD becomes about −10 mV. **b** and **c** The field-induced oscillation in the TEPD measured in the modulation stage at about 6th second and 16th second, respectively, where the field frequency is increased. Be noticed that the averaged TEPD is about −1 mV in (**b**), the same as that without the field application, and finally increased to about −10 mV in (**c**). **d** Averaged values of individual oscillating pulses or the averaged TEPD. Due to the field application, the averaged TEPD is gradually bent to negative direction. 16 s field application raised the TEPD from −1 mV to about −10 mV

from the PCT. At the beginning without the field application (Fig. 1a), the lumen potential is about −1 mV with respect to the peritubular fluid. This value is consistent with the results from the previous studies (Katz and Lindheimer 1975; Jorgensen 1980).

The mechanism of the potential difference measured across the epithelium of renal tubules has been well studied (Katz and Lindheimer 1975; Jorgensen 1980; Planelles et al. 1983; Koeppen and Giebisch 1983). It is not a diffusion potential like the membrane potential determined by the ionic concentration gradients. Indeed, large amount, about two thirds of 145 mM Na^+ ions, is reabsorbed out of the PCT segment. However, the intra-lumen ionic concentrations of Na^+ and K remain unchanged (Feraille and Doucet 2001). The lumen Na^+ ions are transported into the epithelium through various co-transporters with HCO_3^-, glucose, amino acids, and carboxylic acids. The ratio of the number of the transported Na^+ ions and the number of other composites is about 1:1 which is similar as the ratio of the initial Na^+ concentration (145 mM) to the concentration of other composites (150 mM).

Even though large amount of composites are reabsorbed in the PCT segment, the osmolarity in the lumen remains a relative constant of 300 mOsm/L throughout the PCT segment. That is because proximal convoluted tubule has an extremely high permeability to water or high conductance (0.2 Ω^{-1}/cm^2) due to abundance of aquaporin water channels in the PCT cell membranes. Whenever solute molecules are transported out off the lumen, proportional water can be passively and quickly transported across the tubular wall.

Indeed, in the proximal tubules fair amount Na^+ ions are reabsorbed by a paracellular shunt pathway through the tight junctions among epithelial cells instead of through the cell cytoplasm (Planelles et al. 1983). The driving force for this pathway is solvent drag developed in response to active bicarbonate absorption or external solution-driving forces. Therefore, similar as the transepithelium pathway, the solutes transported with water through the paracellular pathway do not affect the osmolarity and the Na^+ concentration in the lumen. Because of both absence of ionic concentration gradient across the epithelium wall and the iso-osmolarity throughout the PCT segment, there is no diffusion potential different across the epithelium.

The transepithelium potential difference in the segment of proximal convoluted tubule has been well documented previously (Katz and Lindheimer 1975; Jorgensen 1980; Koeppen and Giebisch 1983), and named as active potential difference (Jorgensen 1980). It mainly represents cations transported from lumen to the peritubular space driven by the Na^+/K^+ pumps multiplying the resistance across the epithelium (Seely and Chirito 1975; Jorgensen 1980; Koeppen and Giebisch 1983), which can be eliminated by ouabain in the capillary vessel (Mernissi and Doucet 1983). The more Na^+/K^+ pumps activated, or the larger amount of Na^+ ions transported across the epithelium, the higher the magnitude of the active potential difference or the TEPD is.

At about the 1.7th second, the SM electric field in forward modulation mode was applied to the kidney for about 16 s (Fig. 1a). The field-induced transepithelial potential oscillates for about 40 mV with respect to the baseline, or the resting TEPD. Due to hundreds of pulses in the 16 s period, the individual pulses cannot be observed in detail, where we can only observe the profile of the TEPD oscillation. The details of the oscillating pulses at about 6th and 16th seconds are shown in Fig. 1b, and c, respectively.

At beginning or within the 2 s of synchronization stage the profile of the oscillating potential remains relatively symmetric with respect to the baseline. Then, in the modulation stage the profile progressively bents to the negative direction in response to the gradual frequency increase until removal of the electric field.

The effects of the field-induced changes in TEPD can be estimated by average of each individual oscillating pulse throughout the field application. The result is shown as the central curve which is redrawn in Fig. 1d. Magnitude of transepithelium potential difference rose from −1 mV to about −10 mV in response to the SM electric field.

Thirty six experiments have been conducted. Due to difference in the positions of the field-application electrodes and their orientation with respect to the measured PCT segment, the magnitude of the electric field varied from 100 to 1,000 mV in order to adjust the field-induced TEPD in the range from 40 to 50 mV. Figure 2 shows the average of the experiments and standard deviation. Left bar is the control without the field application while the central bar is the result from the forward-modulation stimulation. The results display that less than 20 s application of the

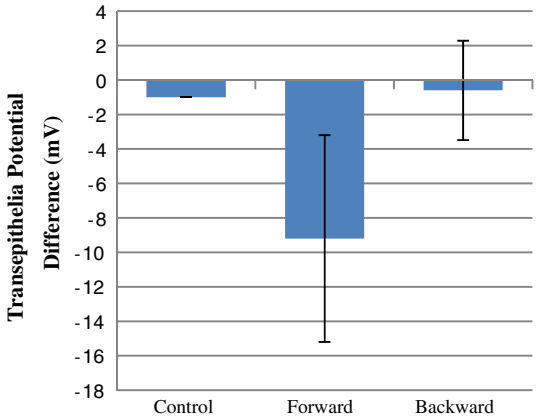


Fig. 2 Statistical results from 41 experiments. Among those, 36 experiments were conducted for the forward-modulation stimulation and five experiments for the backward-modulation. *Left* column is the control without the field application. *Central* column represents the results from the forward-modulation stimulation while *right* column the backward-modulation. The *bars* are the standard deviation. Except the frequency-modulation is opposite all the field parameters and the application protocol are the same. For comparison, the results for the forward- and backward-modulations are shown in the same figure

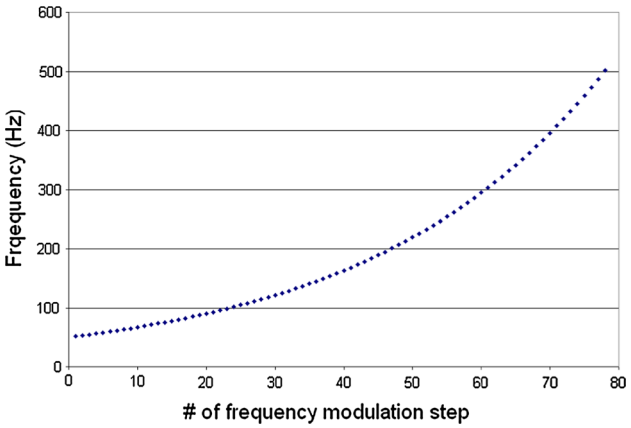


Fig. 3 Frequency change of the SM electric field in the modulation stage, plotted as a function of the number of modulation steps. The frequency is exponentially increased as a function of the step changes

forward SM can significantly increase the transepithelial potential difference in proximal renal tubules.

The increase in the magnitude of transepithelium potential difference in response to the SM electric field can be qualitatively explained by activation of the Na^+/K^+ pumps. During the 2-s synchronization stage, the 50 Hz electric field which is comparable to the pumps' natural turnover rate only force the pump molecules to run at the same pace as the oscillating electric field, while the pumping rate or the number of Na^+ ions transported remains unchanged. Therefore, the averaged TEPD within the first 2 s has little change. Once the pump molecules are activated in the modulation stage running faster and faster, and more and more Na^+ ions are transported, the magnitude of TEPD becomes higher and higher. This result is consistent with our previous results in study of membrane potential in smooth muscle cells of intact peripheral blood vessels (Zhang et al. 2012). The membrane potential was gradually hyperpolarized only during the modulation stage in responding to the SM electric field.

However, the detailed time-courses in the change of potential difference during the modulation stage are different from the smooth muscles we studied (Zhang et al. 2012). For the membrane potential in smooth muscle, the change is very little during early stage of the modulation, and the change becomes greater and greater toward the end of modulation. When plotted as a function of time in the modulation stage, the potential change shows very shallow slope at the beginning and becomes steeper and steeper throughout the field application (Zhang et al. 2012). That phenomenon can be well explained by the field frequency changes in the modulation stage: 3 % increment for every 20 pulses from 50 Hz up to 500 Hz. That means the pumping rate takes 400 ms to increase 3 % in the first step while only take 40 ms in the last step. The curve of the membrane potential change can be well fitted by the field frequency change, which confirmed that the field-induced membrane potential hyperpolarization is due to activation of the Na^+/K^+ pumps.

However, here for the renal tubules the TEPD raised mainly during the early stage of the frequency modulation (Fig. 1d). This is inconsistent with the changes in the field frequency. This is probably because the TEPD is active potential difference not equilibrium potential and the conductance of renal tubule wall also changes corresponding to the ions transport changes.

Based on the initial frequency of 50 Hz and 3 % of the frequency step-change, the field frequency can be written as a function of the number (N) of the modulation steps.

$$f_N = 50 * (1 + 0.03)^N \quad (1)$$

Based on this equation, the field frequency is plotted as the number of modulation steps shown in Fig. 3. At early steps, the frequency increases only slightly, and the frequency change becomes faster and faster showing sharper and sharper slope of the curve. Since the pump molecules were synchronized to the oscillating electric field during the synchronization stage, the above equation also represents the pumping rate during the modulation stage. Due to the lumen iso-osmolarity, whenever solute molecules are transported across the epithelium, proportional water molecules will also be dragged through the tubular wall. Consequently, the wall conductance should also increase proportionally to the ions transported, as shown

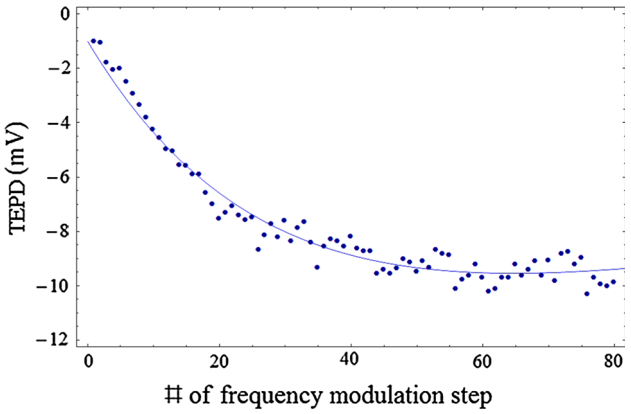


Fig. 4 Re-plotting the field-induced TEPD changes during the modulation stage shown in Fig. 1d, as a function of the number of modulation steps. The potential changes mainly occur at the early stage of the modulation. The curve fitting of the Eq. 3 to the TEPD changes is shown as the continuous curve. See the text

$$g_N = A * 50 * (1 + 0.03)^{B(N-n)} \tag{2}$$

where factor A is a proportional factor from the Na^+ ions transported to the wall conductance. Even though, the wall conductance should be globally or macroscopically proportional to the ions transported, monotonically increasing as a function of the modulation step, the detailed changes in responding to each individual modulation step may be different, such as effectiveness and delay, which we represent by B and n, respectively. Therefore, in response to the field modulation steps or the acceleration of the Na^+ transport, the transepithelium potential can be expressed

$$V_{\mathrm{TE}} = -1 - \frac{\Delta I_{ion}}{\Delta g_{epi}} = -1 - \frac{50 * (1 + 0.03)^N - 50}{A * 50 * (1 + 0.03)^{B(N-n)} - g_o} \tag{3}$$

where -1 represents the control value of the TEPD. The numerator and denominator represent the field-induced changes in the ion transports and the tubular wall conductance, respectively. The "$-$" sign of the second term is because activation of the Na^+/K^+ pump driving the lumen side more negative. The initial pumping rate is 50 Hz and the corresponding conductance is g_o.

As we mentioned above, Fig. 1d is a plot of the averaged transepithelium potential as a function of time. In order to fit the curve by the step of frequency modulation, we can redraw the averaged TEPD as a function of modulation steps, as shown in Fig. 4. Then, we fitted Eq. 3 to the data with a restriction that parameters A *and* B are positive values. The best fitting curve is shown in Fig. 4. The relatively good fitting indicates that the raise in the transepithelium potential difference is related to the frequency change of the modulation electric field or the gradual activation of the Na^+/K^+ pumping rate.

Indeed, many other factors may have minor influence on the transepithelium potential difference. When the Na^+/K^+ pumping rate in the basal lateral membrane is accelerated extruding more Na^+ ions to the peritubular space, if the number of $HCO3^-$ is not enough co-transported to the epithelial cells, more Na^+ ions may be transported than other composites. Therefore, even the luminal osmolarity remains the same the Na^+ concentration may become lower than that at the peritubular fluid. As a result, a small diffusion potential with lumen negative may be generated by the ionic concentration gradient. In addition, Rising of Cl^- concentration in the PCT segment due to its poor permeability may also facilitate the generation of the lumen-negative diffusion potential (Schild et al. 1988). Even so, the relatively well fitting indicates that the TEPD change is mainly due to activation of the Na^+/K^+ pumps by the SM electric field.

Next step, we would like to further confirm that the transepithelium potential change is mainly due to the pumping rate acceleration entrained by the SM electric field. In order to do so, we re-applied the SM electric field with the same waveform, magnitude as well as the application protocol to the kidney except the modulation modes. First, we used constant-frequency mode where the frequency was kept at 50 Hz throughout the field application. The field-induced changes in the transepithelium potential difference were measured and are shown in Fig. 5. Throughout the field application, the profile of the field-induced TEPD is symmetrical with respect to the baseline without bent. The averaged value of individual oscillating pulses is shown as a middle line in the figure which remains the same as the baseline regardless of the field application.

One argument is that 50 Hz is too low which is comparable to the pump natural turnover rate so that the 50 Hz oscillating electric field cannot activate the pump functions. We further applied the electric field in the constant-frequency mode of 500 Hz, which is the same as the end frequency of the SM electric field. The field-induced oscillating transepithemium potential is shown in Fig. 6. Again, the averaged TEPD from individual oscillating pulses remains the same as the value in the control without the field application. Our whole-cell patch clamp experiments in direct measurement of the Na^+/K^+ pumps have shown that simply application of a high frequency of 500 Hz oscillating electric field cannot activate the pump function (Chen et al. 2008). Instead, by first synchronizing the pump molecules to run at the same pumping pace and then gradually modulate their pumping rate, the pump molecules can be accelerated up to 10 times quickly in 10 s. The result of TEPD measurement in renal tubules is

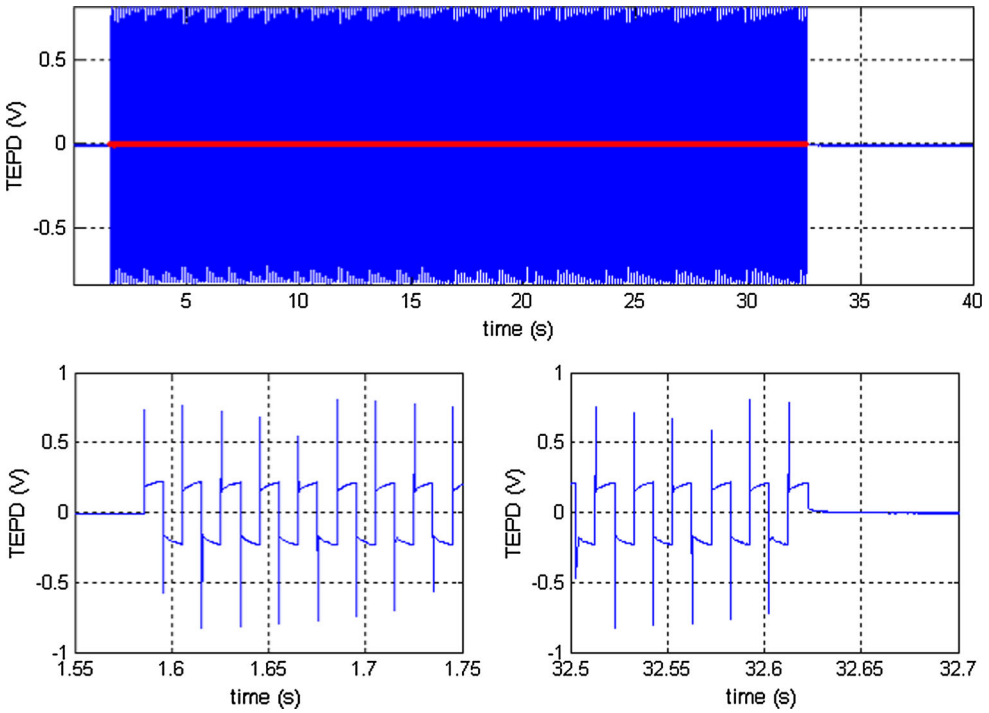


Fig. 5 TEPD in response to the application of the SM electric field in the constant-frequency mode (50 Hz). The detailed oscillations in the TEPD at the beginning and end of the field application are shown in the *lower left* and *right* panels, respectively. The averaged values of individual oscillation pulses show a *relative straight line*, the same as that without the field application, which indicates that the SM electric field without frequency modulation cannot change the TEPD

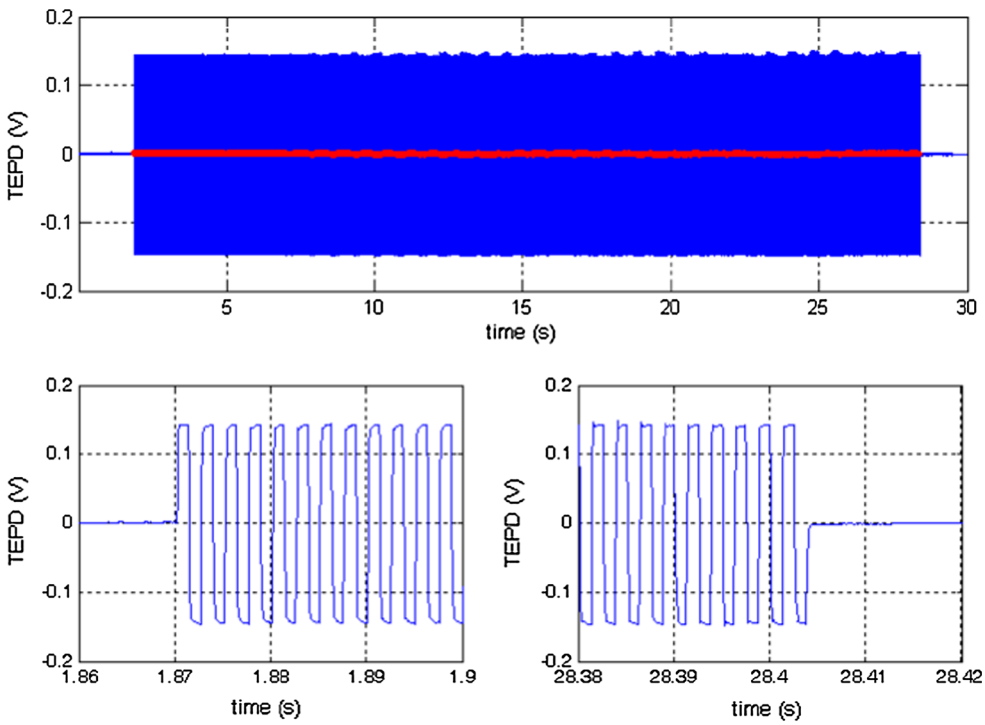


Fig. 6 The field-induced TEPD when exposing to the SM electric field in the constant-frequency mode of 500 Hz, which is ten times higher than the pumps' natural turnover rate. Again, there is no effect on TEPD

consistent with our previous whole-cell patch clamp studies in measurement of the pump current and the membrane potential measurements in blood vessel smooth muscles, which further confirm that the underlying mechanism involved in the pump SM is gradual entrainment of the pump molecules in a stepwise pattern.

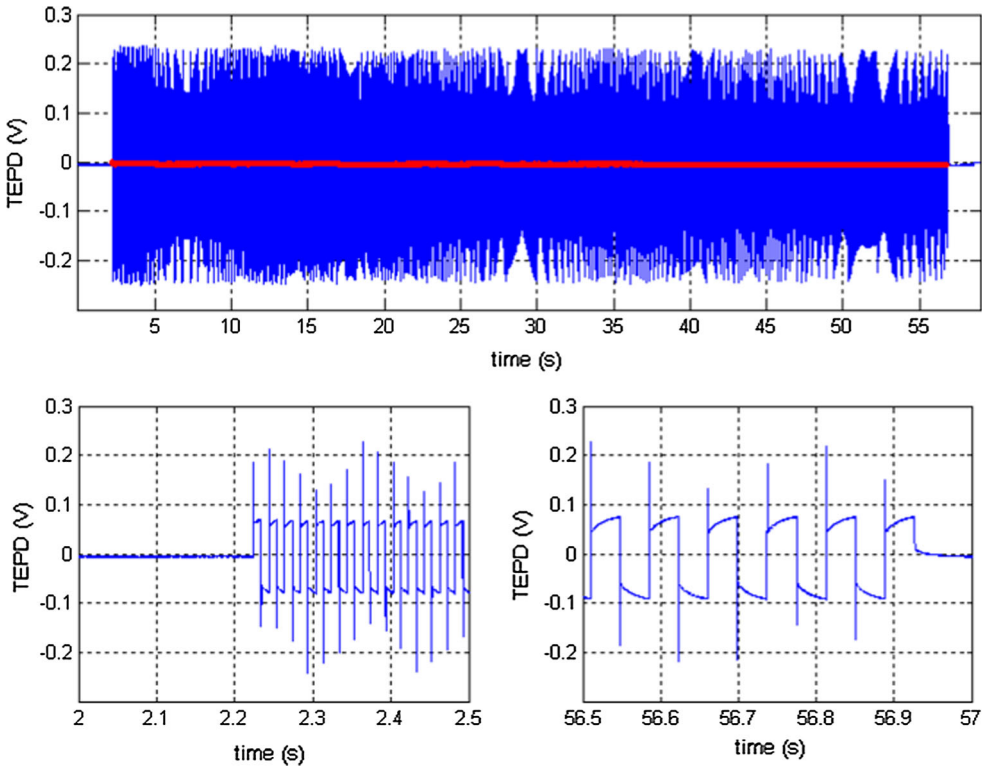


Fig. 7 The measured TEPD in response to the backward modulation SM electric field, starting from an initial frequency of 50 Hz and ended at 10 Hz. Little change is noticeable on the average TEPD

Finally, we applied the SM electric field in the backward modulation to the kidney with the same waveform, magnitude, and field-application protocol. Again, the field initial frequency was 50 Hz. In the modulation stage, the frequency was gradually reduced down to 10 Hz. The field-induced the transepithelium oscillation potential difference is shown in Fig. 7, where the central line represents the averaged values of individual oscillating pulses, which is about the same as that before the field application. The profile of the field-induced potential oscillation remains symmetric with respected to the baseline without bent to the negative direction.

Five experiments were conducted for the backward-modulation stimulation, and the statistic results were obtained. In order to compare the effects on the TEPD from the forward-modulation stimulation, the results are shown as the right bar in Fig. 2.

It is noticed that the backward modulation stimulation only slightly reduced the magnitude of the TEPD and the standard deviation is large. That is because the natural value of the transepithelium potential difference is small only about -1 mV at normal pumping rate. In response to the forward modulation stimulation, ten-time increment in the pump current results in significant increment in the TEPD which can be easily observed. However, even though the pump molecules are fully inhibited, the possible potential change is only about 1 mV. Backward modulation did not block the pumps only reducing the pumping rate. In addition, due to the small value of TEPD, junction potential change may affect the accuracy of the measurement. Therefore, the standard deviation is large.

Discussions

This in vivo study of renal tubules in rat kidney shows that the SM electric field can effectively increase the lumen potential or the transepithelium potential difference in the PCT. The transepithelium potential difference represents the ion flux across the epithelium driven by the Na^+/K^+ pumps. Activation of the pump molecules increases the transports of Na^+ ions, and therefore, other composites from lumen to the petritubular space, which results in larger value of the transepithelium potential difference.

The results are consistent with those obtained previously in our lab in study of the membrane potential of smooth muscle cells in mesenteric blood vessels of rat (Zhang et al. 2012), and those obtained in other lab in study of the transepithelium potential difference across the cultured monolayer of MDCK cell (Tran et al. 2013). All these results indicates that the SM electric field, by activation of the Na^+/K^+ pumping rate, can effectively influence downstream effects in the cell functions, increasing the potential difference, the membrane potential, or the transepithelium potential difference of renal tubules.

In renal tubules, Na^+ ions enter the epithelium across the luminal membrane by coupling various co-transport processes. Na-glucose co-transport, Na-amino acid co-transport,

Na–P co-transport, and Na–H exchange are all associated with the Na^+ transport, or driven by the electrochemical potential energy stored in Na^+ concentration gradient. Significant acceleration of the Na^+/K^+ pumping rate and effectively increase in the transepithelium potential difference indicate that a larger amount of Na^+ ions (much more than the control value at physiological conditions) may be transported across the epithelium wall. Therefore, more efficiency in the reabsorption functions in renal tubules of kidney is expected.

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