

## Influence of the Sodium Transport Inhibition by Amiloride on the Transmesothelial Resistance of Isolated Visceral Sheep Peritoneum

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*The peritoneal mesothelium is a barrier to ion transport in peritoneal dialysis. In the present study, we used Ussing chamber experiments to investigate the effect of amiloride on the transmesothelial electrical resistance ( $R_{TM}$ ) of isolated visceral sheep peritoneum.*

*Peritoneal samples from the omentum of adult sheep were isolated directly after the death of the animals and were transferred to the laboratory within 30 minutes in a cooled Krebs–Ringer bicarbonate solution (4°C, pH 7.5) bubbled with 95% O<sub>2</sub>/5% CO<sub>2</sub>. A visceral peritoneal planar sheet was mounted in an Ussing-type chamber and amiloride (10<sup>-3</sup> mol/L) was added apically and basolaterally. The  $R_{TM}$  was measured before and serially for 30 minutes after the addition of amiloride. Because active ion transport is temperature dependent, the Ussing chambers were held at 37°C. The results presented are the means ± standard error of 12 experiments.*

*The control  $R_{TM}$  (before the addition of amiloride) was 21.86 ± 0.46 Ω · cm<sup>2</sup>. Basolateral addition of amiloride induced, within 1 minute, an increase in  $R_{TM}$  to 27.26 ± 0.39 Ω · cm<sup>2</sup>, a level that persisted throughout the experiment. When amiloride was added apically, the results were similar, with a rapid rise of  $R_{TM}$  to 24.18 ± 0.9 Ω · cm<sup>2</sup> and subsequent value persistence (p < 0.05). A clear association between  $R_{TM}$  and active ion transport was shown in previous studies.*

*The results of the present study indicate rapid action of amiloride on the permeability of the visceral peritoneum. The observed increase in the  $R_{TM}$  indi-*

*cates the existence of amiloride-sensitive sodium channels in the visceral peritoneal membrane. The clinical implications of these results should be further investigated.*

### Key words

Peritoneum, sodium channels, transmesothelial resistance, Ussing

### Introduction

The peritoneal mesothelium is one of the main barriers to water and ion transport from the peritoneal cavity to the peritoneal capillary bed (1). The physiologic transport of solutes across the peritoneal mesothelium is essential for effective peritoneal dialysis (PD) treatment. One of the major problems associated with PD is ultrafiltration (UF) failure, which can affect up to the 50% of PD patients treated for more than 6 years (2,3). It has been proven that peritoneal permeability to small solutes increases with time on PD, a situation that eventually leads to UF failure and PD drop-out (2,3).

Several studies performed in Ussing chambers have shown a clear association between transmesothelial electrical resistance ( $R_{TM}$ ) and transcellular active ion transport in serosal membranes such as peritoneum (4–8) or pleura (9–11). In those studies, membrane permeability alterations were investigated in relation to the action of certain substances (for example, sex hormones, insulin, channel blockers, NO inhibitors, catecholamines, and antibiotics and their metabolites).

Amiloride is mainly an inhibitor of epithelial Na<sup>+</sup> channels. The existence of amiloride-sensitive sodium channels has previously been shown in several

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epithelia—for example, rabbit distal colon, alveolar type II cells, frog lung epithelial cells, rat proximal tubule cells, fetal distal lung epithelial cells, rabbit cecum (12–19)—and in mesothelia such as pleura (9,11), where amiloride was found to inhibit transcellular sodium transport.

In the present study, we examined the inhibitory effects of amiloride on the  $R_{TM}$  of isolated visceral sheep peritoneum. To our knowledge, the effect of amiloride on the  $R_{TM}$  of the peritoneal membrane has scarcely been investigated.

### Materials and methods

Intact sheets of visceral peritoneum were obtained from the omentum of 12 adult sheep (males and females). The samples were collected from the slaughterhouse and transferred to the laboratory in oxygenated Krebs–Ringer bicarbonate (KRB) solution at 4°C within 30 minutes of the death of the animals. Immediately after the peritoneal tissue was removed from the animals, it was placed in the KRB solution, which was balanced at pH 7.4 and bubbled with 95% O<sub>2</sub>/5% CO<sub>2</sub>. The solution contained 117.5 mmol/L NaCl, 1.15 mmol/L NaH<sub>2</sub>PO<sub>4</sub>, 24.99 mmol/L NaHCO<sub>3</sub>, 5.65 mmol/L KCl, 1.18 mmol/L MgSO<sub>4</sub>, 2.52 mmol/L CaCl<sub>2</sub>, and 5.55 mmol/L glucose. The pieces of visceral peritoneum were obtained from the base of the greater omentum. They were carefully isolated from areas with underlying adipose tissue by using a scalpel to remove fat. They were then examined visually for evidence of holes or adherent tissue. The surfaces of the tissue were touched as little as possible.

Visceral peritoneum specimens were carefully mounted in Ussing chambers (Dipl.-Ing. K. Mussler Scientific Instruments, Aachen, Germany) with an opening surface area of 1 cm<sup>2</sup>. Tissues were bathed in 4 mL of KRB solution on each side of the membrane, continuously oxygenated with 95% O<sub>2</sub>/5% CO<sub>2</sub> circulated by gas lift. Two pairs of Ag/AgCl electrodes monitored the transmucosal potential difference (TPD) in millivolts and the  $R_{TM}$  in ohms per square centimeter under open-circuit conditions. The two parameters TPD and  $R_{TM}$  were measured every 6 seconds under existing clamp conditions. Experiments were conducted simultaneously in three computer-controlled chambers (Clamp version 2.14 software: AC Micro-Clamp, Aachen, Germany). Transmucosal electrical parameters were measured in the basal

state (that is, at the end of an equilibration time of 30 – 40 minutes), and during incubations with amiloride apically and basolaterally. After the addition of amiloride 10<sup>-3</sup> mol/L, maximal changes in the  $R_{TM}$  were expressed as the difference from the baseline measurement ( $\Delta R_{TM}$ ). Because active transport of ions is influenced by temperature, the Ussing chambers were held at 37°C.

The experimental solution bathing the surface of the peritoneum that *in vivo* faced the peritoneal fluid is herein called the serosal solution, and the solution bathing the surface that *in vivo* was exposed to the blood supply is herein called the mucosal solution. The mesothelial cell membrane that *in vivo* faced the peritoneal fluid is herein called the apical membrane, and the membrane that *in vivo* faced the blood supply is herein called the basolateral membrane.

An equal number of experiments ( $n = 12$ ) were conducted adding KRB–amiloride solution (10<sup>-3</sup> mol/L) to the serosal and to the mucosal solutions. All solutions were freshly prepared before each experiment, heated to 37°C, and bubbled continuously with a 95% O<sub>2</sub>/5% CO<sub>2</sub> gas mixture. The results presented here are the mean of the 12 separate experiments.

After the addition of amiloride to each bathing solution (mucosal and serosal consecutively), measurements were taken over a period of 30 minutes (at minutes 1, 3, 5, 10, 15, 20, 25, and 30). The voltage responses to applied current pulses of a given amplitude (50  $\mu$ A) and duration (200 ms) were measured. The  $R_{TM}$  was calculated by automatically deducting the initially measured resistance of the solution.

Statistical analyses were performed using the SPSS 10.0 software (SPSS Inc., Chicago, IL, U.S.A.). All data are expressed as mean  $\pm$  standard error. The probability of error for comparison of the mean values was calculated using the *t*-test for paired data. Values of  $p < 0.05$  were regarded as significant.

### Results

The spontaneous electrical potential difference across the visceral peritoneum was not significantly different from zero ( $0.4 \pm 0.1$  mV). Before the addition of amiloride, the  $R_{TM}$  of the peritoneum was found to be  $21.86 \pm 0.46 \Omega \cdot \text{cm}^2$ .

Within 1 minute after the addition of amiloride (10<sup>-3</sup> mol/L) apically, the  $R_{TM}$  increased significantly to  $24.18 \pm 0.9 \Omega \cdot \text{cm}^2$  ( $p < 0.05$ ). After the first minute,

the  $R_{TM}$  remained significantly higher than the control value throughout the observation period of 30 minutes (Figure 1).

After addition of amiloride basolaterally, the  $R_{TM}$  also increased significantly to  $27.26 \pm 0.39 \Omega \cdot \text{cm}^2$  ( $p < 0.05$ ) during the first minute and then declined, although it remained significantly higher than the basal level (Figure 2).

On comparison of the  $R_{TM}$  in the first minute after the addition of amiloride basolaterally with the  $R_{TM}$  after the addition of amiloride apically, a statistically

significant difference was observed ( $p < 0.05$ ). The effect of amiloride on the  $R_{TM}$  was more pronounced basolaterally.

## Discussion

In patients on PD, the functional integrity of the peritoneal membrane is pivotal to the success of the treatment. Understanding the physiology of the membrane is important if fluid UF is to be improved and solute removal is to be optimized.

In the present study, we used recognized electrophysiologic techniques to examine the ionic resistance and transepithelial potential of visceral sheep peritoneal mesothelium. These techniques permitted evaluation of two important parameters: TPD and  $R_{TM}$ . The potential difference across the mesothelium suggests the presence of net ion transport (20). Electrical resistance is a measure of transepithelial ionic permeability, because electrical currents are carried by ions in aqueous solution.

Our data show very low ohmic resistance and no measurable spontaneous potential difference. The  $R_{TM}$  values ( $21.86 \pm 0.46 \Omega \cdot \text{cm}^2$ ) measured in this study lie among the values reported for "leaky" epithelial tissues such as renal proximal tubule, rabbit gallbladder, and sheep pleura (9).

Amiloride is an inhibitor of epithelial sodium channels. One minute after the addition of amiloride, both apically and basolaterally, the ohmic resistance of the visceral sheep peritoneum rose significantly. These findings indicate that the peritoneal mesothelium becomes less permeable to ionic currents after the action of amiloride. The physiologic basis is specifically attributed to the inhibition of transcellular sodium transport. The increase in the  $R_{TM}$  after the addition of amiloride suggests the existence of amiloride-sensitive sodium channels in both apical and basolateral visceral peritoneum.

In several previous studies, amiloride was found to exert similar effects on epithelial tissues (9–11, 16–19). In our study, the increase in  $R_{TM}$  was greater when amiloride was placed basolaterally ( $p < 0.05$ ). That finding leads us to hypothesize that more sodium channels may be present basolaterally in the membrane.

## Conclusions

The results of the present study indicate that amiloride has a rapid effect on the  $R_{TM}$  of visceral sheep

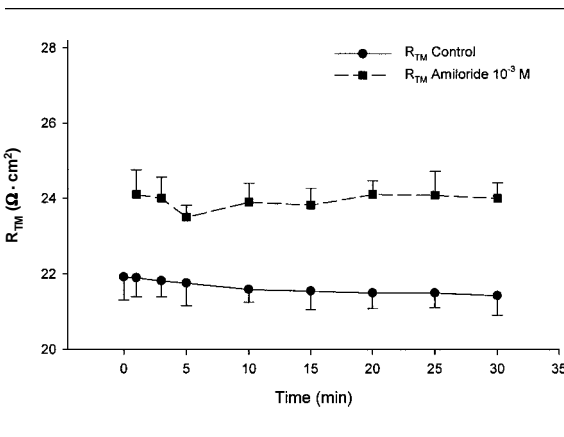


FIGURE 1 The transmesothelial resistance [ $R_{TM}$  ( $\Omega \cdot \text{cm}^2$ )] of the visceral peritoneum before (closed circles) and after addition of amiloride [ $10^{-3}$  mol/L (M)] apically (closed squares). Values are mean and standard error of 12 experiments.

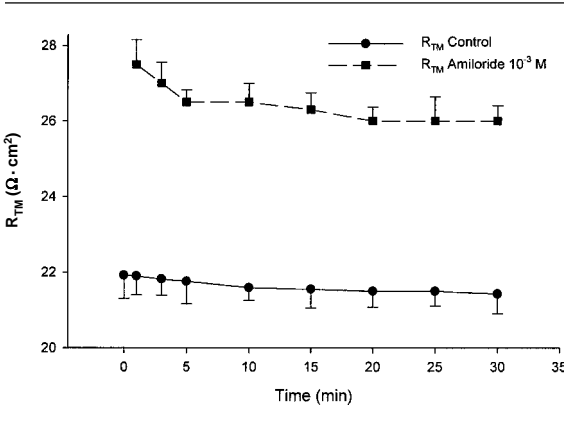


FIGURE 2 The transmesothelial resistance [ $R_{TM}$  ( $\Omega \cdot \text{cm}^2$ )] of the visceral peritoneum before (closed circles) and after addition of amiloride [ $10^{-3}$  mol/L (M)] basolaterally (closed squares). Values are mean and standard error of 12 experiments.

peritoneum whether added apically or basolaterally. A clear association between the  $R_{TM}$  and active ion transport was shown in previous studies (8–11). These facts suggest the ubiquitous existence of amiloride-sensitive sodium channels in the visceral peritoneal membrane. More studies are needed to elucidate the physiologic role suggested by these findings and particularly its clinical significance.

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