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Effect of Sodium–Potassium Pump Inhibition by Ouabain on the Permeability of Isolated Visceral Sheep Peritoneum

Sotirios Zarogiannis,[1] Vassilios Liakopoulos,[2] Chryssi Hatzoglou,[1] Panagiota Kourti,[2] Konstantinos Vogiatzidis,[1] Spyros Potamianos,[3] Theodoros Eleftheriadis,[2] Konstantinos Gourgoulianis,[4] Paschalis–Adam Molyvdas,[1] Ioannis Stefanidis[2]

The permeability for small solutes and the ultrafiltration capacity of the peritoneum are essential for effective peritoneal dialysis (PD) treatment. Elucidation of the factors that regulate these two properties is therefore of great importance. Ouabain, a potent inhibitor of the Na^+–K^+ pump has been shown to reduce fluid absorption in animal models of PD. In the present study, we used Ussing chamber experiments to investigate the effect of ouabain on the transmesothelial electrical resistance (R_{TM}) of isolated visceral sheep peritoneum.

Peritoneal samples from the omentum of adult sheep were isolated immediately after the deaths of the animals and were transferred to the laboratory in cooled Krebs–Ringer bicarbonate solution (4°C, pH 7.5) bubbled with 95% O_2 / 5% CO_2. A planar sheet of visceral peritoneum was mounted in an Ussing-type chamber, and ouabain (10^{-3} mol/L) was added apically and basolaterally. The R_{TM} was measured before and serially for 30 minutes after the addition of ouabain. Because active ion transport is temperature-dependent, all measurements were taken at 37°C. The results presented are the mean ± standard error of 6 experiments.

Before the addition of ouabain, the control R_{TM} was measured as 21.26 ± 0.57 Ω•cm². Addition of ouabain basolaterally induced an increase in the R_{TM} to 27.62 ± 0.72 Ω•cm² within 1 minute ($p < 0.05$), and this level persisted throughout the experiment. The effect of ouabain, when added apically, was similar, characterized by a rapid rise in the R_{TM} to 24.66 ± 0.76 Ω•cm² at 1 minute ($p < 0.05$), with subsequent persistence at that level.

A clear association between R_{TM} and active ion transport has been shown in previous studies. The results of the present study, showing a rapid effect of ouabain on the R_{TM} of visceral peritoneum, therefore clearly suggest that cell membrane Na^+K^+–ATPase is important for peritoneal ionic transport. In addition, ouabain was previously shown to reduce vasodilation and intraperitoneal sodium or to increase intraperitoneal volume, especially in the presence of conventional acidic solutions. Those findings, combined with the results of the present study, clearly indicate that intraperitoneal administration of digitalis glycosides may have some beneficial effect in PD patients; however, the specific clinical implications need further investigation.

From: Departments of [1]Physiology, [2]Nephrology, [3]Gastroenterology, and [4]Respiratory Medicine, Medical School, University of Thessaly, Larissa, Greece.

Key words
Ouabain, peritoneal permeability, sodium–potassium pump, Ussing chamber

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). Physiologic solute transport across the peritoneal mesothelium is essential for effective peritoneal dialysis (PD) treatment. Peritoneal permeability to small solutes has been proved to increase with time on PD, a fact that eventually leads to ultrafiltration (UF) failure and PD dropout (2,3). Ultrafiltration failure is a major problem that can affect up to the 50% of PD patients treated for more than 6 years (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), pleura (9–11), and

pericardium (12). In those studies, permeability alterations were investigated in relation to the action on the membrane of substances such as sex hormones, insulin, channel blockers, NO inhibitors, catecholamines, opioids, and antibiotics and their metabolites.

Ouabain, a cardiac glycoside, binds to high-affinity sites of the cell membrane Na^+–K^+ pump, directly inhibiting its action. In several previous studies, ouabain was found to modulate transcellular ion transport by inhibiting Na^+K^+–ATPase on various epithelia such as lung and urinary bladder (13–15) and mesothelia such as pleura (9,16) and peritoneum (17). Furthermore, ouabain has been shown to reduce peritoneal fluid absorption in rats undergoing PD (18).

Our objective in the present study was to examine the influence of ouabain on the transmesothelial resistance (R_{TM}) of isolated visceral sheep peritoneum. To our knowledge, the interaction between the ouabain-induced Na^+–K^+ pump inhibition and the R_{TM} of peritoneal membrane has been little investigated.

Materials and methods

Intact sheets of visceral sheep peritoneum were obtained from the omentum of 6 adult sheep (males and females). The samples were collected from the slaughterhouse immediately after the deaths of the animals (time of warm ischemia close to 0 minutes). Immediately after removal, the peritoneal tissue from the animals was placed in oxygenated Krebs–Ringer bicarbonate (KRB) solution at 4°C and transferred to the laboratory within 30 minutes. The KRB solution was balanced at pH 7.4 and bubbled with 95% O_2 / 5% CO_2. The solution contained 117.5 mmol/L NaCl, 1.15 mmol/L NaH_2PO_4, 24.99 mmol/L $NaHCO_3$, 5.65 mmol/L KCl, 1.18 mmol/L $MgSO_4$, 2.52 mmol/L $CaCl_2$, and 5.55 mmol/L glucose. All pieces of visceral peritoneum were obtained from the base of the greater omentum. They were carefully isolated from areas with underlying adipose tissue by removal of the fat with a scalpel, and they were then examined for evidence of holes or adherent tissue by visual inspection. The surfaces of the tissue were touched as little as possible.

Specimens of visceral peritoneum were carefully mounted in Ussing chambers (Dipl.–Ing. K. Mussler Scientific Instruments, Aachen, Germany) with an opening surface area of 1 cm^2. Tissues were bathed with 4 mL of KRB solution on each side of the membrane, continuously oxygenated with 95% O_2 / 5% CO_2 circulated by gas lift. Two pairs of Ag/AgCl electrodes monitored the transmesothelial potential difference (in millivolts) and the R_{TM} (in ohms per square centimeter) under open circuit conditions. The two parameters were measured every 6 seconds under current clamp conditions. Experiments were conducted simultaneously in 3 chambers controlled by a personal computer (Clamp software version 2.14). Transmesothelial electrical parameters were measured in the basal state (that is, after an equilibration time of 30 – 40 minutes) and during incubation with ouabain added apically and basolaterally. Because active ion transport is influenced by temperature, all measurements were taken at 37°C.

The experimental solution bathing the surface of the peritoneum that *in vivo* faces the peritoneal fluid is referred to here as the serosal solution, and the solution bathing the surface that *in vivo* is exposed to blood supply is referred to here as the mucosal solution. The mesothelial cell membrane facing the fluid side is here called the apical membrane, and that facing the blood side is called the basolateral membrane.

A total of 12 experiments were conducted, 6 in which KRB–ouabain (10^{-3} mol/L) was added to the serosal solution, and 6 in which KRB–ouabain (10^{-3} mol/L) was added to the mucosal solution. All solutions were freshly prepared before each experiment, heated to 37°C, and bubbled continuously with 95% O_2 / 5% CO_2. The results presented here are the mean of the 6 separate experiments for each case.

After the addition of ouabain 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 response to applied current pulses of 50 μA amplitude and 200 ms duration was measured. The transmesothelial resistance was calculated by automatically deducting the resistance of the solution.

Statistical analysis was performed using SPSS 10.0 for Windows (SPSS, Chicago, IL, U.S.A.). All data are expressed as mean ± standard error. The probability of error for comparisons of 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 very low

(0.46 ± 0.07 mV). Before the addition of ouabain, the R_{TM} of the peritoneum was found to be 21.26 ± 0.57 Ω•cm^2.

At 1 minute after the addition of ouabain (10^{-3} mol/L) apically, the R_{TM} increased significantly to 24.66 ± 0.76 Ω•cm^2 ($p < 0.05$). After minute 1, the R_{TM} remained significantly higher than the control value throughout the 30-minute observation period (Figure 1).

After addition of ouabain basolaterally, the R_{TM} also increased significantly to 27.62 ± 0.72 Ω•cm^2 ($p < 0.05$) by minute 1. Subsequently, it showed a tendency to decline, but it nevertheless remained significantly higher than the baseline level throughout the experiment (Figure 1).

The increase in the R_{TM} was statistically significantly greater when ouabain was added basolaterally than when it was added apically ($p < 0.05$, Figure 1).

Discussion

In the present study, we investigated visceral peritoneal mesothelium from sheep by using recognized electrophysiologic techniques to evaluate two important parameters: transmesothelial potential and transmesothelial resistance. The potential difference across the mesothelium suggests the presence of net ion transport (19). Electrical resistance is a measure of transepithelial ionic permeability because ions in aqueous solution carry electrical currents (19).

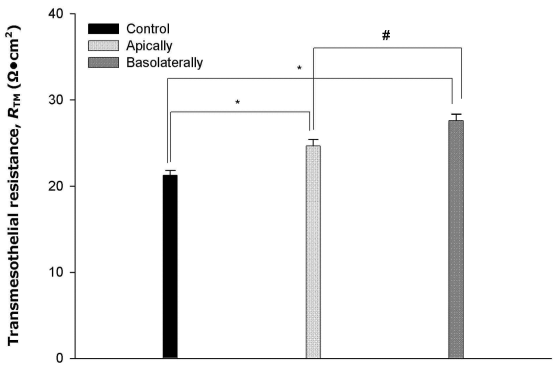

FIGURE 1 The transmesothelial resistance, R_{TM} (in ohms per square centimeter), of the visceral peritoneum (control before the addition of ouabain), 1 minute after addition of ouabain [10^{-3} mol/L (M)] apically, and 1 minute after addition of ouabain basolaterally. Values are mean and standard error of 6 experiments. * $p < 0.05$ vs. control; # $p < 0.05$ basolaterally vs. apically.

Our data showed very low ohmic resistance and no measurable spontaneous potential difference. The R_{TM} values (21.26 ± 0.57 Ω•cm^2) that we observed were similar to values reported for various "leaky" epithelial tissues such as the renal proximal tubule, rabbit gallbladder, and sheep pleura (9).

The Na^+–K^+ pump is a highly conserved, integral membrane ion pump that consumes 1 molecule of ATP to transport 3 Na^+ ions from the interior of the cell to the outside and 2 K^+ ions from the outside to the cell interior. Because of its biochemical characteristics, the Na^+-K^+ pump is also known as Na^+K^+–ATPase. A large body of evidence has established that Na^+K^+–ATPase is responsible for maintaining the transmembrane K^+ gradient that underlies resting membrane potential and the transmembrane Na^+ gradient that, in turn, is essential to all cellular Na^+-dependent transmembrane transport systems. Ouabain is an inhibitor of Na^+K^+–ATPase.

Within 1 minute after the addition of ouabain, either apically or basolaterally, the ohmic resistance of visceral sheep peritoneum rose significantly. This finding indicates that the peritoneal mesothelium becomes less permeable to ionic currents after the action of ouabain. This finding is attributable to the physiologic inhibition of transcellular potassium transport. Furthermore, the increase in R_{TM} after the addition of ouabain suggests the existence of Na^+K^+–ATPase (Na^+–K^+ pump) on both the apical and basolateral sides of the visceral peritoneum. Several previous studies in epithelial tissues found that ouabain exerted such an influence (13–17).

Addition of ouabain raises the R_{TM} of visceral peritoneum within 1 minute. This rapid effect is compatible with direct inhibition by ouabain of the Na^+–K^+ pump. The increase in the R_{TM} is significantly greater when ouabain is added basolaterally than when it is added apically. That finding may be readily attributed to the presence of more Na^+K^+–ATPase on the basolateral side of the mesothelial cell membrane than on the apical side.

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 for improving fluid ultrafiltration and optimizing solute removal. A previous *in vivo* animal study suggested that intraperitoneal use of

ouabain during PD decreases the peritoneal fluid absorption rate (leading to enhanced intraperitoneal volume) and simultaneously increases the peritoneal transport of sodium (18). Conventional PD solutions, which are acidic and hyperosmolar, induce vasodilatation (20). Addition of ouabain to these dialysates was found to reduce vasodilation and to increase intraperitoneal volume (18). A fall in dialysate sodium was attributed mainly to the increased intraperitoneal volume; however, the action of ouabain on transmesothelial ion transport, as clearly demonstrated in our study, could have been an additional contributing factor. Neutralization of dialysis solution *per se* does not completely restore vasodilatation (20). It could therefore be speculated that intraperitoneal addition of ouabain might be beneficial in clinical PD.

Conclusions

Inhibition of the Na^+–K^+ pump has a rapid effect on the transmesothelial resistance (R_{TM}) of the visceral peritoneum. Therefore, cell membrane Na^+K^+–ATPase on the visceral peritoneal mesothelium may be important for peritoneal ionic transport. Ouabain-induced alterations were similar, but more pronounced after addition of ouabain on the basolateral side as compared with the apical side of the membrane. That difference indicates a ubiquitous, but uneven, distribution of Na^+K^+–ATPase on the mesothelial cell membrane. The clinical implications of our results need to be further investigated.

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Corresponding author:
Ioannis Stefanidis, MD, Associate Professor of Nephrology, Division of Nephrology, University of Thessaly, School of Medicine, Papakyriazi 22 str., Larissa 41222 Greece.
E-mail:
stefanid@med.uth.gr