

# Effect of Endothelin-1 on the Transmesothelial Resistance of Isolated Visceral Sheep Peritoneum

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*The mesothelium is part of the peritoneal barrier that manages the water and ion transport essential for peritoneal dialysis (PD) treatment. In addition, it has a central role in the pathogenesis of peritoneal fibrosis and the resulting ultrafiltration failure observed in many PD patients. Endothelin-1 (ET-1) is a potent vasoactive peptide originally described as an endothelial cell-derived factor. In addition, ET-1 has been shown to stimulate fibrogenic activity in various organs by regulating the production and turnover of matrix components.*

*The aim of the present study was to investigate, by means of Ussing chamber experiments, the effect of ET-1 on the transmesothelial electrical resistance ( $R_{TM}$ ) of isolated visceral sheep peritoneum.*

*Intact sheets of visceral sheep peritoneum were obtained from 12 adult sheep. The samples were collected from the slaughterhouse immediately after the deaths of the animals and, within 30 minutes, were transferred in oxygenated Krebs–Ringer bicarbonate (KRB) solution at 4°C to the laboratory to be mounted in an Ussing-type chamber. Endothelin-1 ( $10^{-7}$  mol/L) was then added to the KRB solution apically or basolaterally, and the  $R_{TM}$  was measured before and serially for 10 minutes after the addition of the ET-1.*

*The control  $R_{TM}$  (before addition of ET-1) was  $22.8 \pm 0.56 \Omega \cdot \text{cm}^2$ . Addition of ET-1 apically significantly increased the  $R_{TM}$  by  $63.82\% \pm 16.93\%$  ( $p < 0.05$ ) within 1 minute. After addition of ET-1 basolaterally, the  $R_{TM}$  also increased significantly by  $90.91\% \pm 57.31\%$  within 1 minute ( $p < 0.05$ ). In both cases, these values persisted throughout the experiment.*

*These results clearly indicate an inhibitory effect of ET-1 on the ionic permeability of visceral sheep*

*peritoneum. The rapid increase in  $R_{TM}$  observed after the addition of ET-1 suggests the existence of endothelin receptors (ET-A or ET-B, or both) on visceral sheep peritoneum. Previous studies demonstrated that ET-1, acting on ET-B receptors, potently inhibits epithelial sodium channels in mammalian cell cultures. Nevertheless, the exact pathways that underlie these findings remain unclear; their elucidation requires further investigation.*

## Key words

Endothelin-1, peritoneal permeability, sodium channels, 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. One of the major problems associated with PD is ultrafiltration (UF) failure, which can affect up to 50% of patients treated with PD for more than 6 years (2,3). Peritoneal permeability for small solutes has been proven to increase with time on PD, eventually leading to UF failure and PD drop-out (2,3). Recent studies (4) have clearly shown that mesothelial cells play a central role in the pathogenesis of peritoneal fibrosis and UF failure. However, the properties of the peritoneal mesothelium have not yet been fully elucidated.

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 (5–8) and pleura (9–10). In those studies, permeability alterations were investigated in relation to the action of certain substances such as sex hormones, insulin, NO inhibitors,

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catecholamines, and antibiotics and their metabolites on the actual membrane.

Endothelin-1 (ET-1), a potent vasoactive peptide originally described as an endothelial cell-derived factor, was first isolated from porcine aortic endothelial cells. It is the original member in a family of related peptides each containing 21 amino acids. Three structurally similar isopeptides called ET-1, ET-2, and ET-3 have been identified. Each endothelin is encoded by a distinct gene and is processed from an inactive precursor via two proteolytic cleavages to generate a biologically active peptide.

Endothelins are synthesized in many cell types, including endothelial, epithelial, fibroblast, and cardiac muscle cells, and they function as autocrine or paracrine factors to regulate various cell processes (11). In various organs, ET-1 has been shown to stimulate fibrogenic activity by regulating production and turnover of matrix components (12).

Two ET receptor types—ET-A and ET-B—have been identified. The ET-A receptors are selective for ET-1 and ET-2; the ET-B receptors bind ET-1, ET-2, and ET-3 with equal affinity. Receptors ET-A and ET-B are both heterotrimeric G protein-coupled receptors (13).

Recent evidence indicates that ET-1 has an inhibitory effect on epithelial sodium channels (14) and may therefore play a pivotal role in the regulation of serosal membrane permeability. The objective of the present study was to examine the effects of ET-1 on the transmesothelial resistance ( $R_{TM}$ ) of isolated visceral sheep peritoneum. To our knowledge, this effect has never previously been investigated.

## Materials and methods

Intact sheets of visceral sheep peritoneum were obtained from 12 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) and, within 30 minutes, were transferred to the laboratory in oxygenated Krebs–Ringer bicarbonate (KRB) solution at 4°C. The KRB solution 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.

Specimens of the visceral sheep peritoneum were carefully mounted in Ussing chambers (Dipl.-Ing. K. Mussler Scientific Instruments, Aachen, Germany) with an open surface area of 1 cm<sup>2</sup>. Tissues were bathed with 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 transmesothelial potential difference (in millivolts) and the transmesothelial resistance ( $R_{TM}$ , in ohms per square centimeter), under open circuit conditions. Those 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 and during incubations with ET-1 apically and basolaterally. After the addition of the KRB solution containing ET-1 (10<sup>-7</sup> mol/L), changes in the  $R_{TM}$  were expressed as the difference from the baseline value. Because active transport of ions is influenced by temperature, transmesothelial electrical parameters were all measured 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.

We conducted 12 experiments adding ET-1 (10<sup>-7</sup> mol/L) to the serosal KRB solution and 6 experiments adding ET-1 (10<sup>-7</sup> mol/L) to the mucosal solution. All solutions used 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 in this study are the means of 6 separate experiments.

After the addition of ET-1 to the mucosal and serosal bathing solutions, measurements were taken over a period of 10 minutes (at minutes 1, 3, 5, 10). The voltage response to applied current pulses of 50 μA amplitude and 200 ms duration was measured. The transmesothelial resistance was calculated, automatically deducting the initially measured resistance of the solution.

All statistical analyses were performed using SPSS 10.0 for Windows (SPSS, 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 control  $R_{TM}$  (before addition of ET-1) was  $22.8 \pm 0.56 \Omega \cdot \text{cm}^2$ . The percentage increase in the  $R_{TM}$  observed within 1 minute after the addition of ET-1 apically was  $63.82\% \pm 16.93\%$  ( $p < 0.05$ ). This increase was sustained for 10 minutes (Figure 1).

After addition of ET-1 basolaterally, the  $R_{TM}$  also increased significantly by  $90.91\% \pm 57.31\%$  within 1 minute and remained significantly above the baseline level ( $p < 0.05$ , Figure 2).

Comparison of the  $R_{TM}$  increases at minute 1 after the addition of ET-1 apically and basolaterally showed no statistically significant difference.

### Discussion

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

In the present study, we used recognized electrophysiologic techniques to investigate the ionic resistance and transmesothelial potential of visceral peritoneal mesothelium from sheep. Use of these techniques allowed us to evaluate two important parameters, transmesothelial potential difference and transmesothelial resistance.

The difference in potential across the mesothelium suggests the presence of net ion transport (15). Electrical resistance is a measure of transmesothelial ionic permeability because electrical currents are carried by ions in aqueous solution. A clear association between  $R_{TM}$  and active ion transport was shown in previous studies (8–9).

The present data show that, after the addition of ET-1, transmesothelial resistance increased, and thus the ionic permeability of peritoneal membrane decreased. This effect of ET-1 on ionic transport can probably be attributed to inhibition of ionic channels. Furthermore, the results indicate that the effect of ET-1 on the  $R_{TM}$  of visceral sheep peritoneum is very rapid, whether the ET-1 is added apically or basolaterally.

This rapid action of ET-1 on the  $R_{TM}$  suggests the existence of ET receptors (ET-A or ET-B, or both)

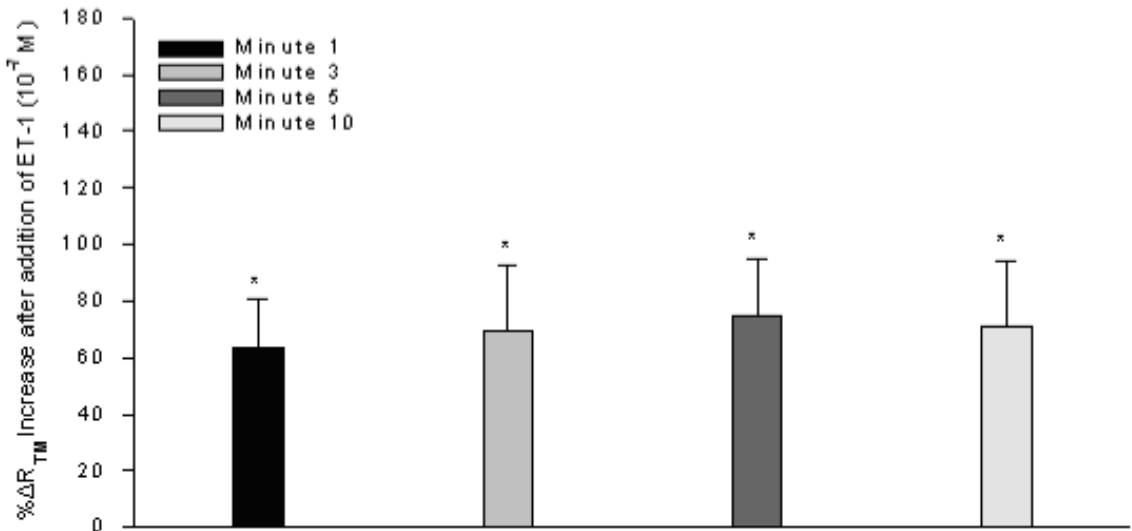


FIGURE 1 Comparison of the percentage change in transmesothelial electrical resistance ( $\% \Delta R_{TM}$ ) of the visceral peritoneum 1, 3, 5, and 10 minutes after addition of endothelin-1 [ $10^{-7}$  mol/L (M)] apically. Values are mean and standard error of 6 experiments. \*  $p < 0.01$  vs. control (baseline).

on both sides of the visceral sheep peritoneum. Previous studies also clearly demonstrated that ET-1, acting on ET-B receptors, potently inhibits epithelial sodium channels in mammalian cells (14). Taken together with previous studies, the present findings suggest that the observed increase in the  $R_{TM}$  is the result of an ET receptor (probably ET-B)-mediated inhibition of sodium channels that have been already shown to exist in mesothelia such as pleura (9–10) and peritoneum (16).

The postulated action of ET-1 on mesothelial sodium channels could potentially be a regulatory mechanism of sodium removal in PD patients with significant clinical implications. Sodium removal during PD dwells has been recognized as a very important aspect of PD treatment, and it is a major determinant of blood pressure control and adequate UF (17). Sodium removal has been shown to be associated not only with better blood pressure control, but also improved survival (18). Many strategies, including prescription of diuretics (19), low-sodium PD solutions, and icodextrin (among others), have been used to improve sodium removal in PD patients. On the other hand, automated PD is known to be characterized by lesser sodium removal

(20) than that seen with continuous ambulatory PD (CAPD). Because the use of automated PD as an alternative to CAPD is rapidly expanding, then all efforts toward a better understanding of the physiologic mechanisms of sodium removal in PD are of great importance.

### Conclusions

Clearly, ET-1 affects the electrophysiologic profile of the peritoneal membrane—that is, it decreases ionic permeability. Furthermore, our results suggest the presence of ET receptors on both sides (apical and basolateral) of visceral sheep peritoneum. The alteration of peritoneal ionic permeability by ET-1 can therefore readily be attributed to an ET receptor-mediated inhibition of mesothelial sodium channels. More studies are needed to elucidate the clinical implications of these findings.

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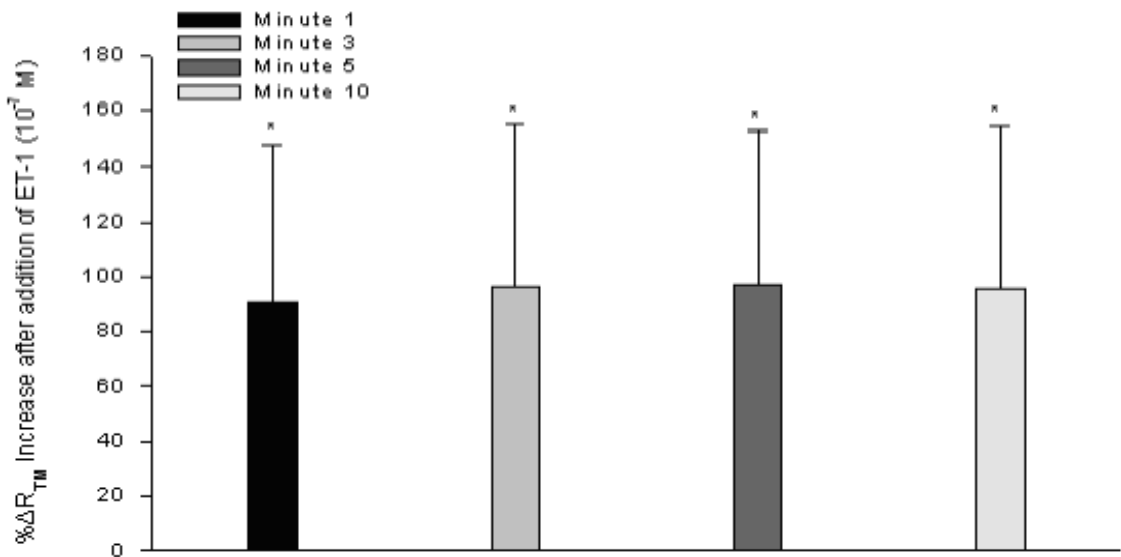


FIGURE 2 Comparison of the percentage change in transmesothelial electrical resistance ( $\% \Delta R_{TM}$ ) of the visceral peritoneum 1, 3, 5, and 10 minutes after the addition of ET-1 [ $10^{-7}$  mol/L (M)] basolaterally. Values are mean and standard error of 6 experiments. \*  $p < 0.01$  vs. control (baseline).

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