

# $\mu$ -Opioid Stimulation of Isolated Parietal Sheep Peritoneum Decreases Peritoneal Permeability *In Vitro*

Sotirios Zarogiannis,<sup>1</sup> Konstantinos Vogiatzidis,<sup>1</sup> Chryssi Hatzoglou,<sup>1</sup> Vassilios Liakopoulos,<sup>2</sup> Spyros Potamianos,<sup>3</sup> Theodoros Eleftheriadis,<sup>2</sup> Spyros Dovas,<sup>2</sup> Panagiota Kourti,<sup>2</sup> Konstantinos Gourgoulialis,<sup>4</sup> Paschalis-Adam Molyvdas,<sup>1</sup> Ioannis Stefanidis<sup>2</sup>

*The peritoneal mesothelium is one of the main barriers to ion transport in peritoneal dialysis. In a previous study, we showed the existence of a  $\mu$ -opioid influence on the in vitro ionic permeability of serosal membranes (specifically, pleura and pericardium), which become less permeable to ionic currents after the action of morphine. In the present study, we used Ussing chamber experiments to investigate the effect of morphine on the transmesothelial electrical resistance ( $R_{TM}$ ) of isolated parietal sheep peritoneum.*

*Peritoneal samples from the diaphragm 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 planar sheet of parietal peritoneum was mounted in an Ussing-type chamber and morphine (10<sup>-9</sup> mol/L) was added apically and basolaterally. The  $R_{TM}$  was measured before and serially for 30 minutes after the addition of morphine. Because active ion transport is temperature dependent, the Ussing chamber was held at 37°C. Results presented are the mean  $\pm$  standard error of 6 experiments.*

*The control  $R_{TM}$  (before the addition of morphine) was  $20.26 \pm 0.57 \Omega \cdot \text{cm}^2$ . Addition of morphine basolaterally induced, within 1 minute, an increase in  $R_{TM}$  of  $24\% \pm 4.8\%$ , which declined thereafter ( $p < 0.01$ ). When morphine was added apically, the results were not similar, because no significant change occurred in the  $R_{TM}$ .*

*The  $R_{TM}$  is an established surrogate of peritoneal permeability. The results of the present study indicate*

*rapid action of basolaterally added morphine on the permeability of the parietal peritoneum. The observed increase in the  $R_{TM}$  indicates the existence in the parietal peritoneum of  $\mu$ -opioid receptors that seem to prevail basolaterally. The clinical implications of these results should be further investigated.*

## Key words

Peritoneal permeability, morphine, transmesothelial resistance, 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). To be effective, the peritoneal dialysis (PD) modality requires physiologic solute transport across the peritoneal mesothelium. Ultrafiltration (UF) failure is one of the major problems associated with PD; UF failure can affect up to 50% of the patients treated with PD for more than 6 years (2,3). The permeability of the peritoneal membrane for small solutes has been proven to increase with time on PD, and this change 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 the peritoneum (4–8), the pleura (9–11), and the pericardium (12). Morphine is a  $\mu$ -opioid receptor agonist and also an opioid analgesic in wide clinical use (13). To date, the literature connects the  $\mu$ -opioid receptors with electrolyte transport (14), and one particular study investigating the effect of morphine on pleura and pericardium revealed that morphine influences the ionic permeability of those

serosal membranes (12). That study found that morphine decreased the ionic permeability of pleura and pericardium alike and that this effect potentially resulted from inhibition of the  $\text{Na}^+ - \text{K}^+$  pump.

Some patients undergoing long-term continuous ambulatory PD (CAPD) require treatment with morphine for pain (15). Conjugation with glucuronic acid represents the major biotransformation route for morphine. The glucuronides morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G) are eliminated via the kidneys. Although systemic clearance of morphine in CAPD patients is in the range observed in patients with normal kidney function, both M3G and M6G show substantial accumulation (15). It is therefore of interest to investigate whether morphine *per se* alters physiologic solute transport across the peritoneal mesothelium.

The aim of the present study was to investigate the effect of morphine on the  $R_{\text{TM}}$ , and thus on the permeability of the parietal peritoneum. To the best of our knowledge, the effect of opioids on the electrophysiologic parameters of the peritoneal membrane has been little investigated.

### Materials and methods

Intact sheets of parietal peritoneum were obtained from the diaphragms 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). After removal, the peritoneal tissue 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%  $\text{O}_2$  / 5%  $\text{CO}_2$ . The solution contained 117.5 mmol NaCl, 1.15 mmol  $\text{NaH}_2\text{PO}_4$ , 24.99 mmol  $\text{NaHCO}_3$ , 5.65 mmol KCl, 1.18 mmol  $\text{MgSO}_4$ , 2.52 mmol  $\text{CaCl}_2$ , and 5.55 mmol glucose. Pieces of the parietal peritoneum were isolated from the diaphragmatic peritoneum and visually examined for holes and adherent tissue. Precautions were taken to avoid touching the surface.

Specimens of the sheep parietal peritoneum were carefully mounted in Ussing chambers (Dipl.–Ing. K. Mussler Scientific Instruments, Aachen, Germany) with an open surface area of 1  $\text{cm}^2$ . Tissues were bathed with 4 mL KRB solution on each side of the membrane, continuously oxygenated with 95%  $\text{O}_2$  / 5%  $\text{CO}_2$  circulated by gas lift. Two pairs of Ag/AgCl

electrodes monitored the transmesothelial potential difference ( $Pd$ , in millivolts) and the transmesothelial resistance ( $R_{\text{TM}}$ , in ohms per square centimeter) under open-circuit conditions. The two parameters  $Pd$  and  $R_{\text{TM}}$  were measured every 6 seconds under current clamp conditions. Experiments were conducted simultaneously in three 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 incubations with morphine added apically and basolaterally. After the addition of morphine  $10^{-9}$  mol, maximal changes in the  $R_{\text{TM}}$  were expressed as the difference from the baseline ( $\Delta R_{\text{TM}}$ ). Because active ion transport is influenced by temperature, measurements of transmesothelial electrical parameters were conducted 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 6 experiments adding morphine ( $10^{-9}$  mol) to the serosal KRB solution, and 6 experiments adding morphine ( $10^{-9}$  M) to the mucosal KRB solution. All solutions were freshly prepared before each experiment, heated to 37°C, and bubbled continuously with a 95%  $\text{O}_2$  / 5%  $\text{CO}_2$  gas mixture. The results presented here are the mean of all the experiments.

After the addition of morphine 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). We measured the voltage response to applied current pulses of a given amplitude (50  $\mu\text{A}$ ) and duration (200 ms). The transmesothelial resistance was then automatically calculated based on the initially measured resistance of the solution.

Statistical analyses were performed using the SPSS software package, version 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 spontaneous electrical potential difference across the parietal peritoneum was very low ( $0.5 \pm 0.11$ ). The basal  $R_{TM}$  of the parietal peritoneum—that is, before the addition of morphine—was  $20.26 \pm 0.57 \Omega \cdot \text{cm}^2$ .

In terms of percentage alteration in  $R_{TM}$  ( $\Delta R_{TM}\%$ ), a significant increase of  $24\% \pm 4.8\%$  ( $p < 0.01$ ) occurred within 1 minute after morphine was added basolaterally, with a decline thereafter, reaching basal values. When morphine was added apically, a slight increase of the  $R_{TM}$  occurred ( $\Delta R_{TM}\%$ :  $8.33\% \pm 5.3\%$ ) that was not statistically significant ( $p = 0.34$ ).

The increase in the  $R_{TM}$  observed in the first minute was greater in the basolateral surface than in the apical surface ( $p < 0.05$ ; Figure 1).

## Discussion

The functional integrity of the peritoneal membrane is pivotal to the success of PD treatment. Understanding the physiology of the membrane is important to improve fluid and solute removal.

In the present study, we used recognized electrophysiologic techniques to investigate the ionic resistance of parietal sheep peritoneal mesothelium after stimulation with morphine. The use of these techniques permitted an evaluation of two important parameters: transmesothelial potential and transmesothelial resistance. The potential difference across the mesothelium suggests the presence of net ion

transport (16). 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 ( $20.26 \pm 0.57 \Omega \cdot \text{cm}^2$ ) measured in this study lie between the values reported for “leaky” epithelial tissues such as the renal proximal tubule, rabbit gallbladder, and sheep pleura (9).

It was demonstrated that the  $R_{TM}$  increased rapidly when morphine was added basolaterally to the parietal peritoneum. That observation indicates the existence of a  $\mu$ -opioid influence on the ionic permeability of the parietal peritoneum, which becomes less permeable to ionic currents after the action of morphine.

Until now, the literature connected the  $\mu$ -opioid receptors with electrolyte transport (14). However, data are rather scarce concerning the influence of morphine on the ionic permeability of serosal membranes. A previous study by our group regarding the effect of morphine on pleura and pericardium showed that the effect is similar to that observed in the present study. We suggested that morphine exerts its action of reducing the permeability of pleura and pericardium probably by inhibition of  $\text{Na}^+ - \text{K}^+$  pump. Given the morphologic and functional resemblance of pleura, pericardium, and peritoneum (17–19) our findings suggest that an existing mechanism of active electrolyte transport through parietal peritoneum is inhibited by the effect of morphine. We speculate that the observed effect could have been mediated by  $\text{Na}^+ - \text{K}^+$  pump inhibition.

Another observation is the difference in the effect of morphine added apically and basolaterally. It can be hypothesized that this finding might be attributed to a difference in the number or subtypes of  $\mu$ -opioid receptors apically and basolaterally in the parietal peritoneum. Another possible hypothesis is an uneven distribution of the  $\text{Na}^+ - \text{K}^+$  pump on the two sides of the mesothelial cell membrane.

Achieving adequate UF is one of the most important goals of PD, and it is one of the major problems related to the continuation of the modality over time. Moreover, regulation of ionic permeability is also a key factor in effective PD. Therefore, understanding the exact mechanisms that regulate this ionic transport could be of great importance. The fact that some patients undergoing long-term CAPD require pain

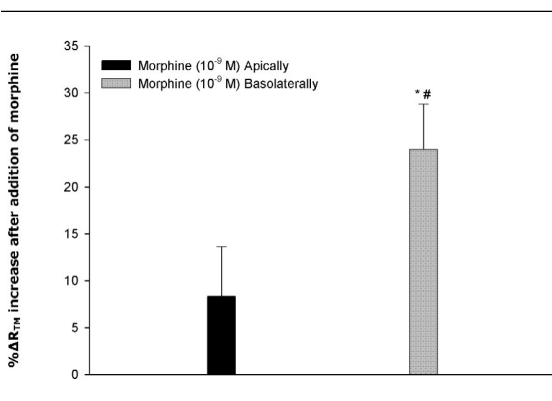


FIGURE 1 Comparison of  $\Delta R_{TM}$  (%) after the addition of morphine [ $10^{-9}$  mol/L (M)] in the parietal peritoneum apically (black error bar) and basolaterally (grey error bar). Values are mean  $\pm$  standard error of 6 experiments. \* =  $p < 0.01$  versus control (baseline); # =  $p < 0.05$  basolaterally versus apically.

treatment with morphine (15), combined with the finding that morphine reduces ionic permeability, should be taken into account in clinical practice.

### Conclusions

Our results indicate the existence of  $\mu$ -opioid receptors in the parietal peritoneum, with basolateral predominance. The effect of morphine basolaterally on the parietal peritoneum is rapid and clear-cut. The physiologic basis of our finding could be induced inhibition of the  $\text{Na}^+$ - $\text{K}^+$  pump by  $\mu$ -opioid receptors. More studies are needed to shed light on the physiologic relevance of our findings and the possible clinical implications.

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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