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# Effect of melittin on *PD*, resistance and short-circuit current in the frog gastric mucosa

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**Abstract**

In an in-vitro preparation of gastric mucosae of *Rana pipiens*, the effect of adding melittin to a concentration of $5 \times 10^{-6}$ M in the secretory solution on the transepithelial potential difference (*PD*), resistance (*R*) and short-circuit current ($I_{sc}$) was studied. In 20 min, melittin decreased the *PD* by 9.3 mV and *R* by 148 ohm cm$^2$. These changes can be explained by a decrease in the resistance, $R_P$, of the paracellular pathway. To determine whether specific-ion pathways were responsible for the decrease in *R*, the effect of melittin on the partial conductances of $Cl^-$, $K^+$ and $Na^+$ was also studied using the ion substitution method. Melittin *decreased* the *PD* response to changes in nutrient $Na^+$, $K^+$ and $Cl^-$ and the *PD* response to changes in secretory $Cl^-$, but did not affect *PD* responses to changes in secretory $Na^+$ or $K^+$. Therefore, melittin decreased the nutrient membrane partial conductances of $Cl^-$, $K^+$ and $Na^+$ and secretory membrane partial conductance of $Cl^-$, without affecting the secretory partial conductances of $Na^+$ or $K^+$. Initially, melittin increased $I_{sc}$ in regular and $Cl^-$-free but not in $Na^+$-free solutions. There was a delayed decrease in $I_{sc}$. The results indicate that melittin decreases $R_P$, increases the $Na^+$ conductance of the secretory membrane and inhibits, eventually, the $Na^+/K^+$-ATPase pump. © 1998 Elsevier Science B.V.

*Keywords:* Membrane potential; Electrical resistance; Short-circuit current; Melittin; Gastric mucosa; (*Rana pipiens*)

## 1. Introduction

Melittin, a 26 amino-acid polypeptide, contained in the honeybee venom [1], has been found to have an active interaction with membranes. It binds to negatively charged phospholipids [2] and has effects on the electrical properties of excitable tissues [3,4]. The venom forms channels in lipid bilayers [5] and also has been found to interact and inhibit the $H^+/K^+$-ATPase [6,7] as well as the $Na^+/K^+$-ATPase [8]. With $10^{-6}$ M melittin in the tear solution, McGahan et al. [9], in the cornea of *Bufo marinus*, and our group [10], in the cornea of *Rana catesbeiana*, found results compatible with an increase in the conductance of the apical membrane of the cornea epithelium. With $10^{-5}$ M melittin in the stromal solution or with $10^{-6}$ M in the tear solution [10], the short-circuit current, $I_{sc}$, was inhibited. This was attributed to an inhibition of the $Na^+/K^+$-ATPase pump located in the basolateral membrane (stromal side). With $3 \times 10^{-7}$ M melittin in the tear solution, the conductance was increased without inhibition of $I_{sc}$ [11]. With $10^{-5}$ M melittin in the nutrient solution of the frog gastric mucosa, melittin inhibited $H^+$ secretion, the

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effect being attributed to an inhibition of the $H^+/K^+$-ATPase pump [12]. However, melittin added to the nutrient solution did not affect the potential difference, *PD*, or resistance, *R*, comparable to similar results with the addition of melittin to the stroma of the cornea [10].

In the present experiments, evidence is presented that, with melittin added to the secretory solution to a concentration of $5 \times 10^{-6}$ M, there is an increase in the paracellular pathway conductance, as well as an increase in the secretory membrane $Na^+$ conductance. No apparent inhibition of the active transport mechanism has been observed.

## 2. Methods

Experiments were performed on fundi of stomachs of *R. pipiens* by an in vitro method in which the stomachs were mounted between a pair of cylindrical chambers [13]. All experiments began with standard $Cl^-$ solutions on both sides of the mucosa, to test the viability of the mucosa. The nutrient (serosal) solution contained (in mM): $Na^+$ – 102; $K^+$ – 4; $Ca^{2+}$ – 1; $Mg^{2+}$ – 0.8; $Cl^-$ – 81; $SO_4^{2-}$ – 0.8; $HCO_3^-$ – 25; phosphate – 1; and glucose – 10; and the secretory (mucosal) solution which is hypertonic [14] contained $Na^+$ – 156; $K^+$ – 4; and $Cl^-$ – 160. For low $Cl^-$ or $Cl^-$-free solutions, $SO_4^{2-}$ replaced $Cl^-$ and sucrose was added to make up any osmotic deficit. For low $Na^+$ or $Na^+$-free solutions, choline replaced $Na^+$. For high $K^+$ solutions, $Na^+$ was replaced by $K^+$. Melittin was added to the secretory (mucosal) solution to a concentration of $5 \times 10^{-6}$ M. Nutrient solutions were used on both sides of the mucosa in experiments performed with symmetrical solutions.

The transmucosal resistance, *R*, and the transmucosal potential difference, *PD*, were measured. Two pairs of electrodes were used, one for sending current across the mucosa and the other for measuring the *PD*. The *PD* is considered positive when the nutrient (serosa) side is positive relative to the secretory (mucosa) side of the stomach. *R* was determined as the change in *PD* per unit of applied current. Current (20 μA per 1.3 $cm^2$ of tissue area) was applied for 1 or 2 s, first in one direction and 2 or 3 s later, in the other direction. $I_{sc}$ was obtained by setting the transepithelial *PD* to zero.

To ensure that the mucosa was actively secreting, the $H^+$ secretory rate was determined by the pH stat method of Heinz and Durbin [15] in the beginning of the experiment, when both sides of the gastric mucosa were gassed with 95% $O_2$/5% $CO_2$ and stimulated with histamine. Then, before addition of melittin and for the rest of the experiment, histamine was washed out and both sides of the gastric mucosa were gassed with 100% $O_2$ to maximize the solubility and effectiveness of melittin at a concentration of $5 \times 10^{-6}$ M. The pH of both solutions with 100% $O_2$ varied from 5.2 to 6.8 in the secretory, and from 8.1 to 8.4 in the nutrient solution. No effects of melittin added to the secretory solution were elicited when both sides of the gastric mucosa were gassed with 95% $O_2$/5% $CO_2$, even at a concentration of $10^{-5}$ M. The pH of both solutions, with these gasses is ca. 5 in the secretory and ca. 7.2–7.3 in the nutrient solution.

The Student's *t* test with paired or unpaired observations was used to determine the level of significance, when applicable.

## 3. Results

### 3.1. Effects of $5 \times 10^{-6}$ M melittin in the secretory solution

Fig. 1 shows graphically the effect on *PD* and *R* of adding melittin to the secretory solution in 38 experiments. The quantitative changes after addition of the venom are presented in Table 1. At 10, 20 and 30 min, the transepithelial *PD* decreased by 9.9, 8.8 and 8.6 mV, respectively, from a control value of 17.9 mV. The respective decreases in *R* were 195, 190 and 185 ohm $cm^2$, from a control value of 285 ohm $cm^2$.

To clarify the need for experiments described in the following, we analyze at this time the decrease in *PD* and *R* induced by melittin. From Fig. 2, we obtain the following equations

$$PD = E_C R_p / (R_p + R_C) \quad (1)$$

$$R = R_C R_p / (R_p + R_C) \quad (2)$$

where *PD* is the transepithelial potential difference; $E_C$, the transcellular *EMF*; $R_p$, the shunt resistance;

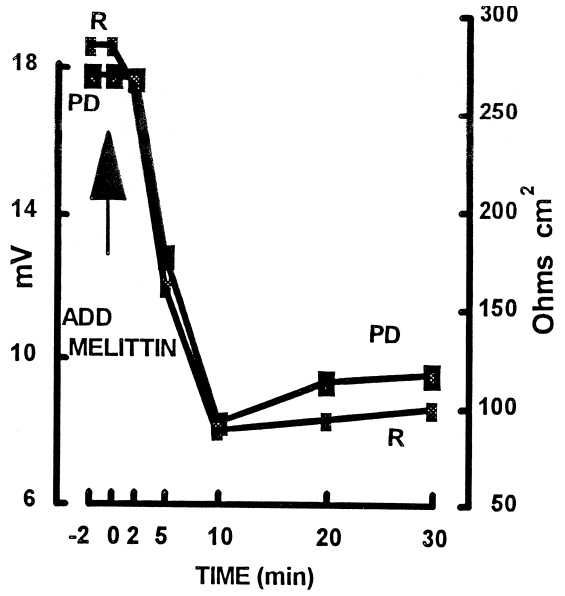


Fig. 1. Effect of $5\times10^{-6}$ M melittin in the secretory solution. *PD*, in mV, and *R*, in ohms cm$^2$, are plotted vs. time. Zero time, when melittin was added

$R_C$, the resistance in series with the EMF; and $R$ the transepithelial resistance.

From Eqs. (1) and (2), the simultaneous decrease in *PD* and *R* is explicable on the basis of a decrease in the parallel resistance, $R_P$, or in combination with a decrease in the EMF, $E_C$. It will be seen below that melittin did not initially decrease $E_C$. A decrease in $R_C$ can also occur but the decrease in $R_P$ predominates.

To determine whether particular ion pathways were involved, we studied the effect of melittin on the partial conductances of $K^+$, $Na^+$ and $Cl^-$ in the basolateral (nutrient) and in the apical (secretory) membranes using the ion-substitution technique.

Table 1
Effects of adding $5\times10^{-6}$ M melittin to the secretory solution[a]

| | Control ($n=38$) | Change in parameter | | |
|---|---|---|---|---|
| | | 10 min | 20 min | 30 min |
| *PD* | $17.9\pm0.9$ | $-9.9\pm0.7$[b] | $-8.8\pm0.5$[b] | $-8.6\pm0.5$[b] |
| *R* | $285\pm10$ | $-195\pm11$[b] | $-190\pm10$[b] | $-185\pm10$[b] |

[a] Values are means + S.E. $n$ = number of experiments. Control values obtained before addition of melittin. The other values are the changes obtained, respectively, at 10, 20 and 30 min after the addition of melittin. Units: *PD* – mV; *R* – ohms cm$^2$.
[b] $P<0.01$.

### 3.2. Effects of $5\times10^{-6}$ M melittin in the secretory solution on the PD response to changes in nutrient $Cl^-$, $K^+$ and $Na^+$ concentrations

Fig. 3 shows graphically the effect on *PD* of changing the $Cl^-$ (upper panel), $K^+$ (middle panel) and $Na^+$ (lower panel) concentration in the nutrient solution before (left panel) and after addition of melittin (right panel) to the secretory solution in eight experiments. The quantitative changes are presented in the upper portion of Table 2.

With a decrease in the *nutrient* $Cl^-$ concentration from 81 to 8 mM, the *transepithelial PD* decreased, at 10 min, by 14.7 mV from a control value of 22.8 mV before melittin and by 6.7 mV from a control value of 9.2 mV with melittin. The *PD* response to the change in nutrient $Cl^-$ concentration was significant without, or with melittin ($p<0.01$). The response was significantly smaller with, than without melittin ($p<0.01$).

With an increase in the *nutrient* $K^+$ concentration from 4 to 79 mM, the *transepithelial PD* decreased, at 10 min, by 27.2 mV from a control value of 20.5 mV without melittin and by 9.0 mV from a control value of 8.1 mV with melittin. The *PD* response to the change in nutrient $K^+$ concentration was significant without, or with melittin ($p<0.01$). The response was significantly smaller with, than without melittin ($p<0.01$).

With a decrease in the *nutrient* $Na^+$ concentration from 102 to 10.2 mM, the *transepithelial PD* decreased, at 10 min, by 4.4 mV ($p<0.01$) from a control value of 19.2 mV before melittin and the *PD* was unchanged with melittin. The *PD* decrease due to a decrease in nutrient $Na^+$ before melittin is ''anomalous'' as it has been repeatedly observed [16–18]. The anomalous *PD* response has been at-

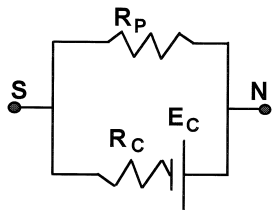


Fig. 2. Equivalent circuit across the gastric mucosa epithelium. $E_C$ is the transcellular *EMF* and $R_C$ the pump resistance. $R_p$ is the resistance of the parallel pathway. $R_p$ includes the paracellular (intercellular) pathway.

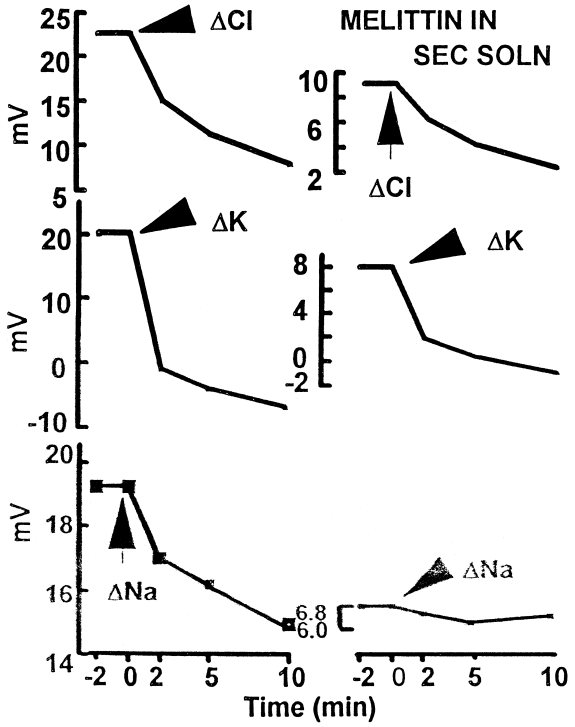


Fig. 3. Effect of changing the concentration of $Cl^-$, $K^+$ and $Na^+$ in the nutrient solutions. Values on the left panel are before melittin and, on the right, after addition of $5\times10^{-6}$ M melittin to the secretory solution. Zero time, when the ion concentration was changed.

tributed to the presence of an electrogenic NaCl [16,18] and/or an $NaHCO_3$ [17,18] symport in the basolateral membrane, transporting more $Cl^-$'s or $HCO_3^-$'s than $Na^+$'s per cycle.

### 3.3. Effects of $5\times10^{-6}$ M melittin in the secretory solution on the PD and R response to changes in secretory $Cl^-$, $K^+$ and $Na^+$ concentrations

Fig. 4 shows graphically the effect on *PD* of changing the $Cl^-$ (upper panel), $K^+$ (middle panel) and $Na^+$ (lower panel) concentration in the secretory solution before (left panel) and after addition of melittin (right panel) to the secretory solution. The quantitative changes are presented in the lower portion of Table 2.

With a decrease in the *secretory* $Cl^-$ concentration from 160 to 16 mM, at 10 min, the *transepithelial PD* increased by 10.2 mV from a control value of 22.8 mV before melittin and by 6.9 mV from a control value of 11.5 mV with melittin. The *PD* response to the change in secretory $Cl^-$ concentration was significant without, or with melittin ($p < 0.01$). The response was significantly smaller with than without melittin ($p < 0.05$).

Table 2
Effects of changing ion concentrations in control, and with $3\times10^{-6}$ M melittin in the secretory solutions [a]

| Ion concentration (mM) | | (*n*) | Control | | Melittin | |
|---|---|---|---|---|---|---|
| initial | final | | *PD* mV | Δ*PD* mV | *PD* mV | Δ*PD* mV |
| Ion concentration change in *nutrient* solution | | | | | | |
| 81 $Cl^-$ | 8 $Cl^-$ | (10) | 22.8 ± 1.3 | −14.7 ± 1.4 [b] | 9.2 ± 1.0 | −6.7 ± 0.8 [b,*] |
| 4 $K^+$ | 79 $K^+$ | (9) | 20.5 ± 1.6 | −27.2 ± 2.3 [b] | 8.1 ± 1.1 | −9.0 ± 1.4 [b,*] |
| 102 $Na^+$ | 10 $Na^+$ | (10) | 19.2 ± 1.5 | −4.4 ± 0.9 [b] | 6.8 ± 1.1 | −0.5 ± 0.3 [c,*] |
| Ion concentration change in secretory solution | | | | | | |
| 160 $Cl^-$ | 16 $Cl^-$ | (10) | 22.8 ± 1.0 | 10.2 ± 1.0 [b] | 11.5 ± 1.0 | 6.9 ± 0.8 [b,**] |
| 4 $K^+$ | 80 $K^+$ | 10) | 22.2 ± 1.6 | −0.4 ± 0.3 [c] | 11.6 ± 1.3 | −1.5 ± 0.9 [c] |
| 156 $Na^+$ | 16 $Na^+$ | (9) | 22.2 ± 1.0 | −4.0 ± 1.0 [b] | 11.0 ± .0.9 | −5.6 ± 1.0 [b] |

[a] Values are means + S.E. n = number of experiments. Control values obtained before the change in ion concentration. Δ*PD* values are the changes in *PD* at 10 min after the change in ion concentration.
Statistic (A): Effect of change in ion concentration:
[b] $P < 0.01$.
[c] $P > 0.05$.
Statistic (B): Effect of melittin on change in *PD*:
* $P < 0.01$.
** $P < 0.05$.
Absence of asterisk, $P > 0.05$.

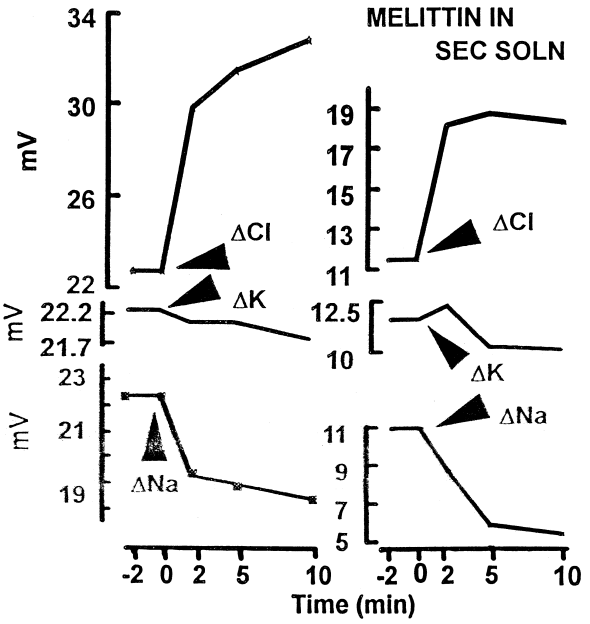


Fig. 4. Effect of changing the concentration of $Cl^-$, $K^+$ and $Na^+$ in the secretory solutions. Format as in Fig. 3.

With an increase in the *secretory* $K^+$ concentration from 4 to 80 mM, the *PD* did not change significantly without, or with melittin. This is contrary to previous observations in which there was a *PD* response to changes in secretory $K^+$ [19,20]. We note that during 100% $O_2$ gassing, without and with melittin there was no histamine present. The absence of histamine and of $CO_2$ in the gas, apparently affected the secretory membrane $K^+$ conductance.

With a decrease in the *secretory* $Na^+$ concentration from 156 to 16 mM, at 10 min, the *transepithelial PD* decreased by 4.0 mV ($p < 0.01$) from a control value of 22.2 mV before melittin and by 5.6 mV ($p < 0.01$) from a control value of 11.0 mV with melittin. Although there appears to be a higher response with than without melittin, the difference was not statistically significant.

The solutions used in the aforementioned experiments are asymmetrical for $Na^+$ and $Cl^-$, with higher concentrations of these ions in the secretory than in the nutrient solution.

In order to evaluate the effect of melittin on the EMF ($E_C$, Fig. 2), $I_{sc}$ was measured in 10 additional experiments. Five experiments were performed as those presented above, that is, with asymmetrical solutions. Another five experiments were performed with nutrient solution on both sides. Data in Table 3 show the effect of melittin on *PD*, *R* and $I_{sc}$ in both groups of experiments. Melittin significantly increased $I_{sc}$ by 8.6 from 37 $\mu A/cm^2$ with symmetrical solutions but did not significantly affect $I_{sc}$ with asymmetrical solutions. Melittin in the secretory solution did not inhibit the transepithelial EMF, $E_C$, responsible for $I_{sc}$.

In both symmetrical and asymmetrical solutions, melittin decreased *PD* and *R*. We should note that *R* in symmetrical solutions is higher than that in asymmetrical solutions. We have previously shown that lowering the NaCl composition in the secretory solution raises *R* [14].

The fact that melittin did not decrease the secretory membrane $Na^+$ partial conductance (see Section 4) and that melittin increased the $I_{sc}$ with symmetrical solutions, could be explained by an increase in the secretory membrane $Na^+$ conductance. To test this possibility, experiments were performed in $Na^+$-free

Table 3
Effects of adding $5 \times 10^{-6}$ M melittin to the secretory solution on $I_{sc}$ [a]

| *PD* mV | Δ*PD* mV | *R* Ohm cm$^2$ | Δ*R* Ohm cm$^2$ | $I_{sc}$ $\mu A/cm^2$ | Δ$I_{sc}$ $\mu A/cm^2$ |
|---|---|---|---|---|---|
| Asymmetrical solutions | | | | | |
| 24.3 ± 2.4 | −13.6 ± 2.9 [b] | 291 ± 12 | −192 ± 21 [b] | 54.6 ± 5.6 | −2.5 ± 3.3 [d] |
| Symmetrical solutions | | | | | |
| 24.8 ± 4.1 | −10.2 ± 0.9 [b] | 545 ± 16 | −340 ± 60 [b] | 37.0 ± 5.8 | 8.6 ± 2.9 [c] |

[a] Values are means ± S.E. No. of experiments = 5 in each case. Δ values are the changes obtained at 10 min after the addition of melittin. See Section 2 for composition of solutions.
[b] $P < 0.01$.
[c] $P < 0.05$.
[d] $P > 0.05$.

Table 4
Effects of adding $5 \times 10^{-6}$ M melittin to the secretory solution on $I_{sc}$ in Na-free or Cl-free solutions [a]

| Symmetrical Na-free solutions ($N = 8$) [a] | | | Symmetrical Cl-free solutions ($N = 7$) [b] | | |
|---|---|---|---|---|---|
| control | Δ(5) | Δ(10) | control | Δ(5) | Δ(10) |
| *PD* (mV) | | | | | |
| 9.2 ± 2.9 | −3.7 ± 1.0 [b] | −6.6 ± 1.4 [b] | 10.3 ± 2.8 | 3.5 ± 1.8 [d] | −5.3 ± 2.1 [c] |
| *R* (Ohm cm$^2$) | | | | | |
| 351 ± 25 | −181 ± 42 [b] | −236 ± 39 [b] | 547 ± 43 | −224 ± 44 [a] | −392 ± 46 [b] |
| $I_{sc}$ (μA/cm$^2$) | | | | | |
| 20.2 ± 6.1 | −6.3 ± 1.1 [b] | −14.5 ± 2.3 [b] | 14.4 ± 4.1 | 17.2 ± 1.3 [b] | 2.4 ± 2.4 [d] |

[a]Values are means ± S.E. N = No. of experiments. Δ – values are the changes obtained at 5 and 10 min after the addition of melittin.See Section 3 for composition of solutions.
[b] $P < 0.01$.
[c] $P < 0.05$.
[d] $P > 0.05$.

solutions. Since $Cl^-$ contributes to $I_{sc}$ under physiological conditions [21], experiments were also performed in $Cl^-$-free solutions.

### 3.4. Effects of $5 \times 10^{-6}$ M melittin in the secretory solution in $Na^+$-free and in $Cl^-$-free solutions

The quantitative changes after addition of the venom are presented in Table 4.In *$Na^+$-free* solutions, with $Cl^-$ present, the *PD* and *R* decreased at 5 and 10 min, as observed in regular solutions. On the other hand, $I_{sc}$ decreased, contrary to observation in regular solutions. In *$Cl^-$-free* solutions, with $Na^+$ present, the *PD* did not change at 5 min, but decreased at 10 min. The *R* decreased markedly at 5 and 10 min. $I_{sc}$ increased markedly at 5 min, but the effect was not significant at 10 min.

The data on $I_{sc}$ are also presented in Fig. 5. The initial effect of melittin is an increase in $I_{sc}$ as long as $Na^+$ is present. The effect is sustained longer in regular solutions than in Cl-free solutions.

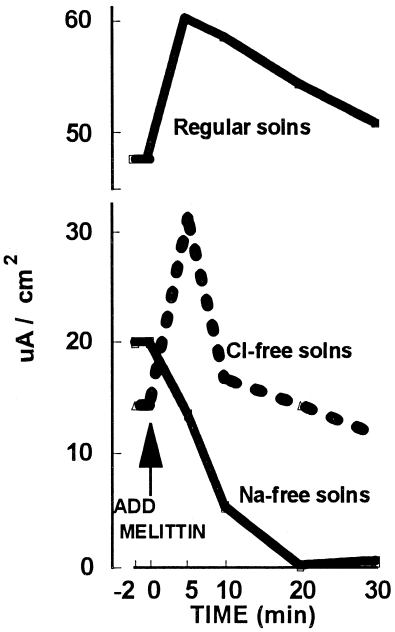


Fig. 5. Effect of $5 \times 10^{-6}$ M melittin in the secretory solution in regular (upper graph), $Cl^-$-free (middle graph) and $Na^+$-free (lower graph) solutions. Zero time, when melittin was added.

## 4. Discussion

The fact that melittin at a concentration of $5 \times 10^{-6}$ M in the secretory solution increased $I_{sc}$ initially in the presence of $Na^+$ suggests that melittin, at this concentration, did not inhibit the transepithelial EMF ($E_C$, Fig. 2) during the initial period. As discussed previously in Section 3, the decrease in *PD* observed with melittin was mostly due to a decrease in $R_P$ (see Eqs. (1) and (2)), that is, melittin increased the conductance of the paracellular pathway. In addition, there is evidence that melittin affected other pathways such as the secretory membrane $Na^+$ conductance, which is included in $R_C$.

Most of the known conductive pathways are presented in Fig. 6: In the nutrient membrane, $Cl^-$

[16–18,22,23], $NaHCO_3$ symport [17,18], $K^+$ [22,24,25], NaCl symport [16–18] and the $Na^+/K^+$-ATPase [25]; and in the secretory membrane, $Cl^-$ [19,20,26,27], $K^+$ [19,20], $Na^+$ (in $Cl^-$-free solutions) [28] and the proton pump [15,29–33].

We have tested the effects of melittin on the conductances of $Na^+$, $K^+$, and $Cl^-$ in both membranes by the ion substitution method.

The *PD* response to a change in the concentration of a particular ion depends on the partial conductance of that ion (see Appendix A). Smaller *PD* responses with melittin, than without melittin, in the secretory solution were observed with changes in the nutrient $Cl^-$, $K^+$, and $Na^+$ concentrations and with changes in the secretory $Cl^-$ concentration. The increase in the paracellular conductance pathway can explain the simultaneous increase in transepithelial conductance, the decrease in the nutrient membrane partial conductances of $Cl^-$, $K^+$, and $Na^+$ and the decrease in the secretory membrane partial conductance of $Cl^-$ (see Appendix A).

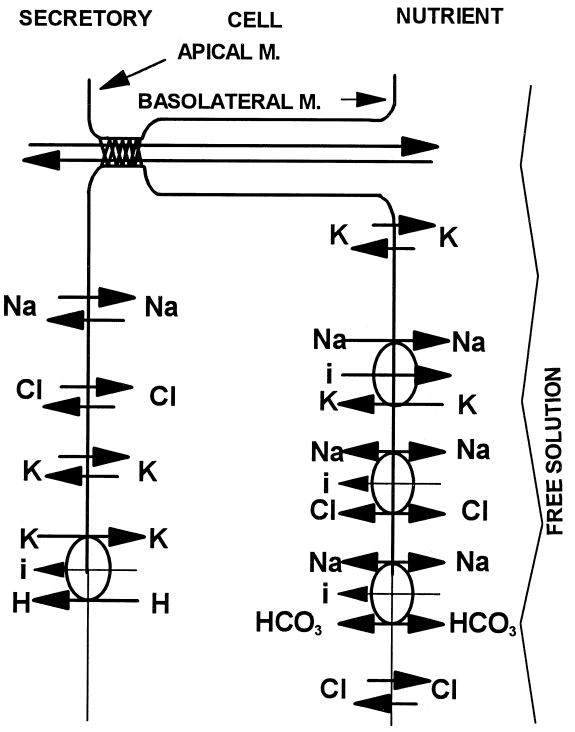


Fig. 6. Schematic representation of the ionic pathways in the secreting gastric epithelium (see text).

With reference to the effects of changing the secretory $K^+$ concentration, we should note the lack of *PD* response in present experiments was contrary to previous observations [19,20]. Present experiments were performed without histamine and bubbling with 100% $O_2$ gas (no $CO_2$) to elicit the effects of melittin described in this paper. No effect of melittin was obtained when bubbling with 95% $O_2$/5% $CO_2$. Previous experiments on $K^+$ [19,20] were performed bubbling with 95% $O_2$/5% $CO_2$. Perhaps the lack of histamine and $CO_2$ affected the secretory membrane $K^+$ conductance. Certainly, melittin did not increase the $K^+$ conductance in the secretory membrane.

The *PD* response to a change in the secretory $Na^+$ concentration was essentially unaltered by melittin. Since the sum of the partial conductances equals one (see Eq. (A.2), Appendix A), a lack of change in the $Na^+$ partial conductance, accompanied by an increase in the transepithelial conductance, suggests that the secretory membrane $Na^+$ conductance must have increased. Further support for the increase in the apical membrane $Na^+$ conductance by melittin is obtained from the experiments performed in $Na^+$-free and in $Cl^-$-free solutions. While in $Na^+$-free solutions, with $Cl^-$ present, there was no increase in $I_{sc}$ (it quickly decreased), in $Cl^-$-free solutions, with $Na^+$ present, the initial (5 min) increase in $I_{sc}$ was similar to the increase in regular solutions.

From the equation $I_{sc} = E_C/R_C$, the initial increase in $I_{sc}$ may be explained by a decrease in $R_C$ which includes the apical membrane $Na^+$ conductance, as mentioned above. However, the initial increase in $I_{sc}$ was reversed with time. The decrease in $I_{sc}$ may be explained by a delayed inhibition of $E_C$ by melittin. We note that the initial increase in $I_{sc}$ was sustained longer in regular solutions than in $Cl^-$-free solutions. In $Cl^-$ solutions, besides the apical $Na^+$ conductance opened by melittin, $Na^+$ is provided to the $Na^+/K^+$-ATPase pump via the NaCl symport.

We further note that there was a finite $I_{sc}$ in $Na^+$-free solutions. The $I_{sc}$ was quickly abolished by melittin. A possible explanation is the presence of residual $Na^+$ in the cell which may leak out into the secretory solution upon addition of melittin.

In summary, the addition of melittin to a concentration of $5 \times 10^{-6}$ M in the secretory solution of the frog gastric mucosa decreases *PD* and *R* but initially

increases $I_{sc}$. The effects on *PD* and *R* can be explained by a decrease in $R_P$. The initial increase in $I_{sc}$ is explained by an increase in the secretory membrane $Na^+$ conductance. A subsequent decrease in $I_{sc}$ suggests that there is a delayed inhibition of the $Na^+/K^+$-ATPase pump.

## Appendix A. Transmembrane potential and ionic EMF's

The change in membrane potential adjacent to the solution in which the change in ion concentration occurs, $\Delta V_m$, is given by

$$\Delta V_m = g^*_{Cl}\Delta E_{Cl} + g^*_K\Delta E_K + g^*_{Na}\Delta E_{Na} + g^*_X\Delta E_X \quad (A.1)$$

where $g^*$'s are the partial conductances of the subscripted ions; and $\Delta E$'s the changes in diffusion potential across the membrane induced by the change in the concentration of the given ion on one side of the membrane. Eq. (A.1) applies to either the nutrient (basolateral) or secretory (apical) membrane, respectively, when the ion concentration is changed in the nutrient or secretory solution, respectively.

We further note that

$$g^*_{Cl} + g^*_K + g^*_{Na} + g^*_X = 1 \quad (A.2)$$

In addition, we observe that the transepithelial *PD* can be presented as

$$PD = V_{NS} = V_{NC} + V_{CS} \quad (A.3)$$

where subscripts N, C and S refer to nutrient, cell and secretory compartments, respectively. $V_m$ (as defined in Eq. (A.1)) will refer to $V_{NC}$ or $V_{CS}$ depending on whether the ions are changed in the nutrient or secretory solutions, respectively.

If melittin increased the conductance of a particular ion, $g_i$, then, $\Delta V_m$ (and $\Delta PD$), induced by the change in the concentration of that ion, would be greater with melittin than without it. On the other hand, an increase in $g_i$ will result in a decrease in the $g^*$'s of ions other than *i* and, therefore, may result in a net decrease in $\Delta V_m$ and $\Delta PD$ induced by the change in the ionic concentration for ions other than *i*.

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