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Effects of melittin on a model renal epithelium, the toad urinary bladder

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The bee venom melittin, 10^{-6} M, on the mucosal (urinary) side of the toad urinary bladder (in vitro), markedly decreased transepithelial potential difference, short-circuit current (I_{sc}, sodium-dependent) and resistance. However, these effects were not seen when the toxin was placed on the opposite (serosal) side of the membrane preparation. The electrical effects were accompanied by a large increase in the transepithelial permeability to ^{22}Na. The response was not changed by meclofenamic acid (which blocks formation of prostaglandins) but it was inhibited by La^{3+}. In the presence of amiloride, which usually inhibits active Na transport and I_{sc}, melittin, on the mucosal side, increased the I_{sc}. The action of melittin appears to involve an interaction with anionic sites, which mediate its effects. Such sites appear to be present on the apical plasma membranes of the toad bladder epithelial cells, but they are not as abundant or they are inaccessible on the basal plasma membrane.

Introduction

Melittin is a cationic amphiphilic peptide, which is present in bee venom and exerts a variety of toxic effects in tissues [1]. Such actions include hemolysis of erythrocytes and increases in vascular permeability. Melittin exerts such effects through its ability to combine with lipophilic and/or hydrophilic groups on natural or even artificial membranes, which results in increases in their permeability [2]. This response may involve the activation of enzymes, such as adenylate cyclase [3] and phospholipase A_2 [4]. The latter effect may result in the formation of prostaglandins [5]. Melittin can be used as a 'membrane probe' in order to characterize such properties of cell membranes [6,7]. In the present study, we describe its effects, in vitro, in the toad (*Bufo marinus*) urinary bladder, which for over 25 years [8,9] has been used as a model renal epithelium. This cellular membrane actively transports sodium and responds by permeability changes to antidiuretic hormone, aldosterone and diuretic drugs [10–12]. It displays transepithelial electrical and ionic permeability properties, which reflect asymmetrical differences between the functioning of the apical and basal plasma membranes of its epithelial cells. We have found that melittin can produce pronounced increases in the ionic permeability from the mucosal (urinary) surface, but not from the serosal (blood) side. These effects are changed by amiloride and lanthanum, both of which can combine with membrane anionic groups.

Materials and Methods

Toad (*Bufo marinus*) were obtained from National Reagents, Bridgeport, CN and kept on damp towels in a room at 21°C.

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The toads were double-pithed and each lobe of the urinary bladder was removed and placed in a Ringer solution containing (mM): NaCl, 111; KCl, 3.3; calcium gluconate, 1.0; $NaHCO_3$, 4.0 and glucose, 5.5. The tissue was then mounted between two lucite Ussing-hemichambers. To reduce 'edge damage', parafilm O-rings were placed between the tissue and the edges of the chambers. A piece of nylon net was suspended across the serosal surface of the bladder tissue to limit flapping movements due to the agitation of the fluids on either side. Each side of the bladder was bathed with 10 ml of the Ringer solution, which was aerated. The exposed surface area of the bladder tissue was 3 cm^2. The experiments were performed at room temperature ($21 \pm 1°C$). In the experiments testing the effects of lanthanum on the bladder, a modified Ringer Tris-buffered solution was used. It contained (mM): NaCl, 103; KCl, 2.5; calcium gluconate, 1.0; Tris, 2.0 and glucose, 5.0.

The transepithelial electrical parameters, potential difference, short-circuit current (I_{sc}) and resistance (R) were measured through a pair of agar-Ringer bridges and calomel cells (for potential difference) and Ag-AgCl cells (for I_{sc}). These were connected to an automatic voltage clamp (Schema Versetae) and a potentiometric recorder. The voltage clamp also automatically recorded the resistance at 40-s intervals by passing a current, in alternate directions, across the membrane for 0.2 s, which was sufficient to change the potential difference by 10 mV. The open circuit potential difference was recorded at appropriate intervals by interrupting the short-circuiting. The electrical resistance of the toad bladder was usually much greater (see Table I) than that of the bathing media (about 40 $\Omega \cdot cm^2$), so that compensation of the applied short-circuit current for the resistance of the external media would be quite small and was not made. These procedures have been described in detail previously [13].

The passive unidirectional flux of Na from the serosal to mucosal side was measured using the isotope ^{22}Na (New England Nuclear, Boston, MA) as a tracer. The I_{sc} was allowed to stabilize and then the isotope (about 0.2 $\mu Ci/ml$) was added to the solution on the serosal side of the bladder. Samples of fluid were then collected at 20–30-min intervals from the media on the mucosal side until a stable movement of isotope was observed (usually 4–5 successive periods). The melittin was then added to the media and collections of fluid continued. The flux was calculated from the counts accumulated and the specific activity of the Na on the serosal side. The ^{22}Na was counted in a gamma-counter (Beckman Biogamma II).

Amiloride was a gift from the Merck Institute for Therapeutic Research, Westpoint, PA and meclofenamic acid from Warner Lambert, Ann Arbor, MI. Melittin was purchased from Sigma, St. Louis, MO.

Results

Effects of melittin on the toad urinary bladder

When melittin in concentrations as low as 10^{-6} M was placed in the solution bathing the mucosal (urinary) side of the toad urinary bladder (in vitro), there was a rapid decline in the transepithelial short-circuit current (I_{sc}, reflecting a net transepithelial Na transport from mucosa to serosa [9]), electrical potential difference and resistance (Fig. 1a, Table I). The potential difference was abolished after about 30 min and the effect was not reversible when a melittin-free solution was subsequently placed on the mucosal side of the preparation. In contrast to this effect, when melittin was placed on the serosal (blood) side of the bladder (10^{-6} M), there is no significant decline in the I_{sc} or potential difference (Fig. 1b, Table I) and, indeed, we sometimes observed a small increase in I_{sc}. The effect of melittin on the mucosal side of the bladder was accompanied by a large increase in permeability to Na. Thus, the unidirectional flux of ^{22}Na (seven preparations) from the serosal to mucosal side increased from 0.21 ± 0.068 to 3.06 ± 1.14 $\mu equiv. \cdot cm^{-1} \cdot h^{-1}$ in the 10 min after addition of the melittin to the media on the mucosal side. The large increase in Na permeability may partly reflect a lysis of some cells, as has been observed in erythrocytes [1], so that paracellular pathways may then facilitate the ion movements. It would appear that in the presence of melittin the bladder is very permeable to the Na and, as the I_{sc} is abolished, a net transport of Na from mucosal to serosal is no longer occurring.

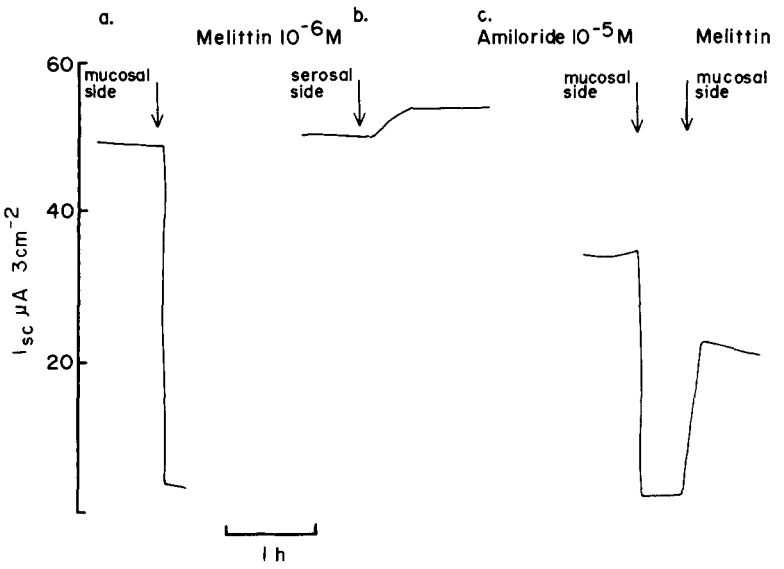

Fig. 1. The effects of melittin (10^{-6} M) on the short-circuit current (I_{sc}) across the toad urinary bladder in vitro. (a) Melittin on the mucosal side; (b) melittin on the serosal side; (c) amiloride, 10^{-5} M, on the mucosal side, and then melittin.

Effects of amiloride and lanthanum on the response to melittin

We tested the effects of two agents, which, like melittin, can interact with anionic sites. Amiloride is a diuretic drug with a cationic guanidinium group, which can combine with such sites on the

TABLE I

EFFECTS OF MELITTIN (10^{-6} M) ON TRANSEPITHELIAL ELECTRICAL PARAMETERS IN TOAD (*B. MARINUS*) URINARY BLADDER IN VIVO

Results are as means ± S.E. for the number of experiments in parentheses. p.d., potential difference; La, lanthanum.

Conditions	(I) Initial [a]			(II) Melittin [b]			Mean difference		
	I_{sc} ($\mu A \cdot cm^{-2}$)	p.d. (mV)	R (ohms-cm^2)	I_{sc}	p.d.	R	I_{sc}	p.d.	R
Melittin on mucosa (7)	16	53	4146	2	1	923	14 ± 4 *	52 ± 10 ***	3223 ± 765 **
Melittin on serosa (7)	22	46	2721	23	46	2449	1 ± 0.5	0.0	272 ± 98 *
Amiloride 10^{-5} M on mucosa in I and II, melittin on mucosa in II (5)	4	9	5005	15	5	475	11 ± 4.3 *	4 ± 1.9	4530 ± 1187 **
La 1 mM on mucosa in I and II, melittin on mucosa in II (7)	9	33	4170	8	22	3079	1 ± 0.4	11 ± 5.8	1091 ± 810
Meclofenamic acid 10^{-5} M both sides in I and II, melittin on mucosa II (6)	9	39	4851	1	1	1258	8 ± 2.0 *	38 ± 11.0 **	3322 ± 655 **

[a] Immediately before adding melittin. [b] Maximal change or approx. 20 min after adding melittin. Amiloride was present for 20 min, La for 60 min and meclofenamic acid for 30 min before adding melittin. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ for mean differences.

apical plasma membrane of toad bladder epithelial cells [14,15] and blocks active Na transport and the I_{sc}. When melittin was added to the solution at the mucosal surface in the presence of amiloride, there was an increase in the I_{sc} (Fig. 1c, Table I). The resistance, however, still declined. Lanthanum ion, La^{3+}, also combines with anionic sites on plasma membranes [16] and in its presence, also on the mucosal side, all of the effects of melittin were blocked (Table I).

Effects of meclofenamic acid on the response to melittin

Prostaglandins can mediate some of the effects of melittin (see Introduction). However, meclofenamic acid, 10^{-5} M, which blocks prostaglandin synthetase [17], did not diminish the action of melittin on potential difference, I_{sc} or resistance across the toad bladder (Table I).

Discussion

Melittin decreases the I_{sc}, potential difference and electrical resistance across the toad urinary bladder in vitro. However, sensitivity of this membrane preparation to the toxin was asymmetrical, as this response was only seen when it was placed in the solution bathing the mucosal side of the tissue, presumably reflecting differences in functioning and composition of the plasma membranes on each side of the epithelial cells that line the bladder. The effect does not appear to be mediated by prostglandins as it was not inhibited by meclofenamic acid and it may be a direct one on the tissue.

The response to melittin can, however, be modified by amiloride and abolished by La^{3+}. Both of these agents, like melittin, can bind to anionic groups. In the instance of amiloride, this involves a cationic guanidinium group, which interacts with such sites that are associated with sodium channels in the apical plasma membrane so that active Na transport and I_{sc} is inhibited [14,15]. Melittin overcomes the inhibitory effect of amiloride and then stimulates the I_{sc}, a response which is accompanied by a decline in resistance. This effect is reminiscent of the action of amphotericin B [14,18], which acts by disrupting sterols in the apical plasma membrane. Sodium, thus, appears to be admitted through nonspecific pathways into the cells, which results in a stimulation of its active transepithelial transport. The reasons for the ability of amiloride to change the nature of the response to melittin are not clear. However, it may be excluding the toxin from some sites of its action, such as sodium channels, so that damage is more discriminate and transepithelial Na transport can be maintained. The regulation of Na transport in the toad urinary bladder may be influenced by kallikrein, which has been identified in this tissue [19]. Amiloride can inhibit the activity of this enzyme, an effect which may be contributing to the former's inhibitory effects on I_{sc} [20]. It is, thus, also possible that melittin may overcome this inhibition of the effects of kallikrein, thus, contributing to the restoration of the I_{sc}. In contrast to amiloride, La^{3+} abolished all electrical effects of melittin on the toad urinary bladder. La^{3+} binds to anionic sites on the plasma membrane, which can be occupied by Ca^{2+}, hence displacing it and/or blocking its exchanges with intracellular Ca [16]. It is possible that the response to melittin is Ca-dependent and this has been shown in other tissues [21,22] where prostaglandin formation may be initiated. However, it is also likely that in this instance the La^{3+} is acting more directly and is also excluding melittin from binding to the anionic sites. Such hydrophilic sites may be essential for the response of the epithelial cells to the toxin and may be more abundant, or accessible, in the apical as compared to the basal plasma membranes. Their presence may be related to the special permeability properties of the apical plasma membrane of the toad bladder epithelial cells.

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