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Leishmania amazonensis Infection Induces Changes in the Electrophysiological Properties of Macrophage-like Cells

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Abstract. Whole cell patch-clamp recordings were used to study the electrical properties of the macrophage-like cell line J774.1, after infection with *Leishmania amazonensis.* Infection induced a significant increase in cell size and membrane capacitance, suggesting that parasite invasion leads to the addition of plasma membrane to the host cell. By 24 hr after infection, the host cell membrane potential was significantly more hyperpolarized than control cells, and this difference remained for the subsequent 72 hr post-infection. The hyperpolarization was paralleled by an increase in the density of inward rectifying K^+ currents. The shape of the conductance *vs.* voltage curve, the kinetic properties and the pharmacological profile of these currents were not significantly altered by infection. These results suggest that infection by *L. amazonensis* causes an increase in the number of functional inward rectifying K^+ channels, leading to hyperpolarization of the host cell membrane.

Key words: Patch-clamp — Macrophages — Parasites — Ionic currents — Capacitance — Membrane potential — K^+ inward rectifier

Introduction

Leishmania species are intracellular obligatory parasites of macrophages. After entry into its mammalian host, the parasite is phagocytosed by macrophages and confined to a lysosome-like compartment (reviewed by Russell, 1995), known as the parasitophorus vacuole (PV). Since the parasites replicate within the PV they must import nutrients from the mammalian host across a series of three barriers: the parasite surface membrane, the membrane of the PV, and the surface membrane of the macrophage.

In several parasite-host systems, the parasite changes the plasma membrane of its host cell by incorporating specific proteins or by modulating constitutive transporters, thus modifying ion homeostasis to secure fast uptake of nutrients and discharge of waste products (Gero & Upston, 1992). In the case of *Plasmodium,* it is known that the parasite increases the transport systems of the erythrocyte membrane for glucose, phospholipids, purine bases, nucleotides, small nonelectrolytes, lactate, anions and cations (reviewed by Ginsburg, 1994; Cranmer et al., 1995). Infection of cardiac mammalian cells with *Trypanosoma cruzi* alters the shape of the action potential by reducing transient outward K^+ currents, changes the basal levels of intracellular Ca^{2+} (Ca_i) and the properties of gap junctions (Campos de Carvalho et al., 1994; Pacioretty et al., 1995). Moreover, changes in Ca_i are considered to play a critical role during host cell invasion by *T. cruzi* (reviewed by Andrews, 1995).

In the case of *Leishmania,* little information is available regarding the impact of parasite invasion on the ion homeostasis of its host cell. There is evidence for increases in basal Ca_i in monocytes and macrophages infected with *Leishmania* (Eilam, El-On & Spira, 1985; reviewed by Olivier, 1996), but changes in the intracellular concentrations or transport properties of other ions have not been reported. Understanding these fundamental aspects of parasite-host interaction is important not only from the cell biological viewpoint, but also for the design of therapeutic agents. In the present work, the whole cell recording configuration of the patch-clamp

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technique (Hamill et al., 1981) was used to explore the effects of infection by *Leishmania amazonensis* on the electrical properties and inward K^+ currents of the macrophage-like cell line J774.1. Our results show that infection has several effects on J774.1 cells. Firstly, it induces changes in membrane capacitance and membrane potential. Secondly, it increases the density of inward rectifying K^+ currents. We describe here the expression of these changes as a function of time after infection.

Materials and Methods

Cell Culture

The murine macrophage-like cell line J774.1 was obtained from the European Cell line and Hybridome Bank Collection (EECACC No. 91051511) and maintained as a monolayer in 25 cm^2 flasks at 37°C, 5% CO_2 for up to 4 weeks. Cells were kept in RPMI 1640 culture medium (GIBCO) supplemented with 10% fetal bovine serum (FBS, GIBCO), 100 U/ml penicillin and 100 μg/ml spreptomycin (GIBCO). Only suspended cells were allowed to attach onto sterile glass coverslips kept in 35 mm petri dishes at 37°C for 24 hr prior to electrophysiological studies. The medium was changed daily and 1 hr before recording.

Parasite Culture and Infection

A *Leishmania amazonensis* isolate kindly donated by Dr. N. Saravia (CIDEIM, Cali, Colombia) was used. Promastigotes, at an initial concentration of 1×10^6, were cultured at 24°C in 25 cm^2 flasks in Schneider's medium (GIBCO) supplemented with 20% FBS. Promastigotes were allowed to reach their metacyclic (infective) stage and either diluted to maintain the culture or harvested for infection. Eighty percent confluent J774.1 cell cultures were exposed to metacyclic promastigotes at a ratio of 1:10 respectively, and incubated at 34°C, 5% CO_2 for 4 hr. Nonadherent promastigotes were washed out with serum-free medium and the cultures were kept for up to 4 days post infection in the same conditions as described above for control cells (Chang, 1981). Infected cells in suspension were allowed to adhere onto sterile glass coverslips and kept in 35 mm petri dishes at 34°C for 24 hr prior to electrophysiological studies.

Electrophysiological Recording and Data Analysis

Coverslips were placed in a recording chamber kept at room temperature (18–20°C) on the stage of an inverted Zeiss IM35 microscope. Cells were bathed in a solution consisting of (in mM): 145 NaCl, 5 KCl, 1 $CaCl_2$, 2 $MgCl_2$, 10 HEPES-Na, 5 glucose (pH 7.34, 300 mOsm). Membrane currents were recorded in the whole cell configuration of the patch-clamp technique with an Axopatch-1C amplifier (Axon Instruments, Foster City, CA). Pipettes were filled with a solution containing (in mM): 140 K glutamate, 2 KCl, 5 EGTA-K, 0.5 $CaCl_2$, 4 $MgCl_2$, 10 HEPES-K, 3 ATP-Na_2, 0.5 GTP-Na (pH 7.34, 300 mOsm). They were made from nonheparinized hematocrit capillaries (Fisher Scientific No. 02-668-6) and had resistances of 2.5–5 MΩ. During whole cell recording the series resistance was not greater than 10 MΩ and it was left uncompensated.

After attaining the whole cell configuration, the amplifier was set to the current-clamp mode to determine the potential at which the current was zero. This value is referred to as resting membrane potential (V_m). Note that, even though the measurement was obtained within the first minute after breaking into the cell, this value may deviate from the V_m of a nondialyzed cell where the cytoplasmic environment has not been altered by the recording-pipette-solution. Throughout the rest of the experiment, the cell was maintained under voltage clamp. Voltage steps were applied from a holding potential of −60 mV. Currents were filtered at 1 kHz and digitized at 200 μsec/point with a DigiData 1200 Interface (Axon Instruments). Data acquisition and analysis were performed with the pclamp6 software (Axon Instruments).

Statistical results are given as mean ± SEM. To compare data from control and infected cells, multiple group comparisons were performed using ANOVA and Dunnett tests; P values ≤ than 0.05 were considered significant. When used, n refers to number of cells included in the analysis.

Results

Changes in Passive Membrane Properties During Infection

Infection with *L. amazonensis* is characterized by the presence of PVs that contain amastigotes, the intracellular stage of the parasite. In our experimental conditions, 48.7 ± 7.4% of the macrophages were infected at 24 hr post infection (pi), as evidenced by the clear appearance of a PV enclosing amastigotes. This PV can be visualized under light microscopy. Figure 1*A* shows a photograph, taken in the recording setup, of a noninfected cell whose cytoplasm is devoid of large vacuoles, and of a 48 hr pi cell where a large PV can be easily recognized. Electron microscopy of the infected cells details the presence of an amastigote within the PV (Fig. 1*B*). Although macrophages with multiple PVs were present in the cultures, we focused our study on infected cells having a single PV, as exemplified in Fig. 1*A*. Throughout the time window of the present work (72 hr pi), the percentage of infected macrophages remained constant. However, the number of amastigotes per cell increased. Thus, up to 48 hr pi there were on average 2.8 ± 0.2 amastigotes per cell ($n = 144$) and by 72 hr pi a two fold increase in parasite number was detected ($n = 60$). We interpret this as indicating that during the first 48 hr pi parasites differentiate and subsequently they start replication.

After 24 hr pi, once the parasite was established within the PV, cells had diameters of 19.2 ± 0.36 μm ($n = 27$), significantly larger than those of controls (16.7 ± 0.28 μm, n = 35). This difference was maintained throughout the time window explored (Fig. 2*A*). Increases in cell size can result from addition of new membrane to the cell plasma membrane or from other mechanisms not requiring membrane recruitment, such as cell swelling and differences in membrane ruffling. Only the former process will yield an increase in the cell membrane capacitance (C_m). Therefore, we compared

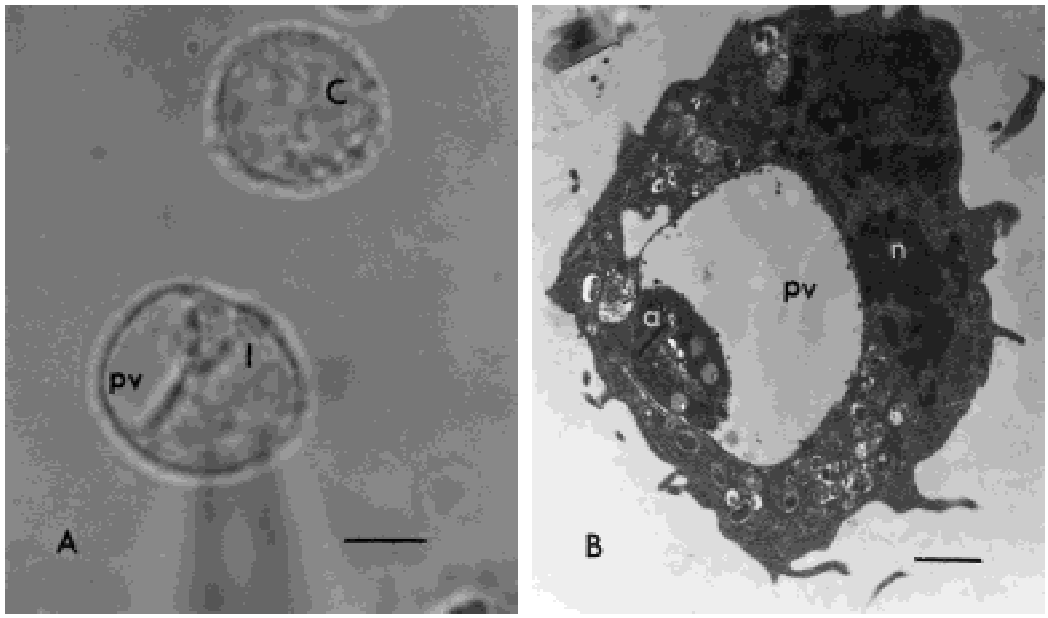

Fig. 1. Changes in J774.1 cell morphology after infection by *Leishmania amazonensis.* (*A*) Photograph of a control cell (*C*) and of a cell at 24 hr pi (*I*), taken under normal light transmitted in the recording setup × 400. Note the presence of a PV (pv) inside the infected cell and the recording pipette (p). The calibration bar = 5 μm. (*B*) Photograph of an electron microscopy image of an infected cell (48 hr pi), which reveals the amastigotes (a) inside the parasitophorus vacuole (pv); *n* denotes the macrophage nucleus. The calibration bar = 1 μm.

C_m in control and infected cells. C_m was read directly from the slow capacitance compensation circuit of the amplifier, a procedure justified by the mono-exponential nature of the current relaxation following a voltage step of −10 mV from the holding potential. Membrane capacitance increased steadily with time after infection (Fig. 2*B*). In all infected groups, C_m was significantly larger than in control cells. At 72 hr pi, C_m values were 48% higher (20.4 ± 1.8 pF, $n = 9$), than the control value (13.7 ± 0.6 pF, $n = 35$). Previous reports had shown that, in J774.1 cells, C_m varies as a function of time of adherence onto glass (Gallin & Sheehy, 1985). Therefore all results presented in this work were gathered from cells plated 24 hr prior to recording, in order to eliminate the possibility that changes could arise from a nonspecific effect of cell-glass interaction. Thus, assuming that the dielectric properties of the lipid bilayer of J774.1 do not change during infection, our data indicate that *L. amazonensis* infection induces the addition of new membrane to the host cell plasma membrane.

We next compared control and infected cells in terms of their resting membrane potential (V_m). As shown in Fig. 2*C*, infection by *L. amazonensis* resulted in a clear hyperpolarization. The average V_m in control cells was −39.4 ± 4 mV ($n = 11$). At 24 hr pi, V_m had already changed by 38% with respect to control and by 48 hr pi the cells had a V_m of −63 ± 2.4 mV ($n = 11$), 58% more hyperpolarized than controls. All infected groups differed significantly from the control cells. We attempted to correlate this hyperpolarization with a change in the cell passive membrane resistance (R_m), by measuring the current response to a 10 mV step from the holding potential, a voltage range where active conductances should be negligible. This parameter failed to reveal any changes. Resistances were 1.8 ± 0.35 GΩ ($n = 7$), 2.1 ± 0.86 GΩ ($n = 13$) and 2.7 ± 1.9 GΩ ($n = 7$) at 24, 48 and 72 hr pi, respectively, and not significantly different from the value for the control group (2.00 ± 0.44 GΩ, $n = 12$). The lack of change can be explained by the fact that the measurement corresponds to the parallel combination of the seal resistance (R_{seal}) and the real membrane resistance (*rRm*). It is thus difficult to detect small deviations from *rRm* when its value is close to that of R_{seal}. Nevertheless, the hyperpolarization induced by infection indicates, a modification in the properties and/or number of ion channels contributing to set the host cell V_m. Therefore, the remainder of this work deals with the effects of infection on a K^+ channel that is considered to play a key role in determining the resting membrane potential of macrophages.

Comparison of Inward Rectifying K^+ Currents in Control and Infected Cells

Several types of ionic currents have been found in monocytes, macrophages and macrophage-like cell lines. Among the K^+ currents described, two are prominent: an inward rectifying current and a depolarization-activated outward current with partial inactivation (Ypey &

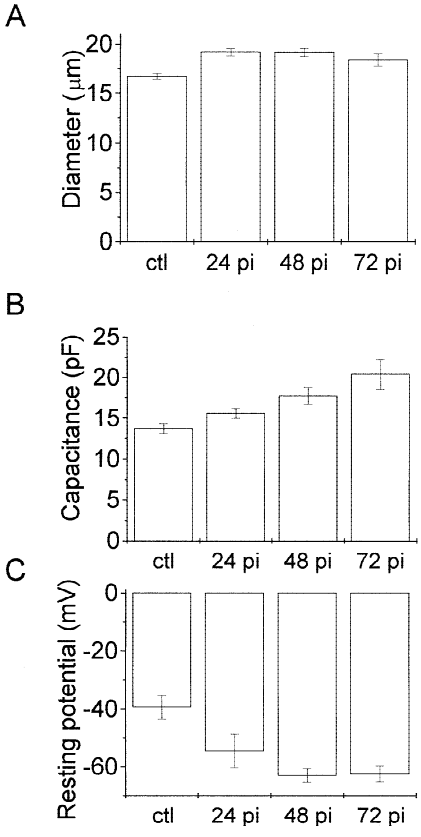

Fig. 2. Effect of *Leishmania amazonensis* infection on cell size, membrane capacitance and resting membrane potential of J774.1 cells. (*A*) Cell diameter was measured with the eye piece micrometer ×400 (*n* was: 35, 27, 24 and 9 for control, 24, 48 and 72 hr pi respectively). (*B*) Values of the membrane capacitance read from the slow capacitance circuit of the patch-clamp amplifier, for the same set of cells used in *A*. (*C*). Resting membrane potential for a subset of the control and infected cells. The value corresponds to the zero current potential measured in current clamp mode within 1 min after attainment of the whole cell configuration (*n* was: 12, 7, 13, and 7 for control, 24, 48 and 72 hr pi, respectively). For the data included in *A*, *B* and *C*, ANOVA tests indicate that all the infected groups differ significantly from the controls at the $P < 0.05$ level. In this and the following figures, the bars correspond to the SEM.

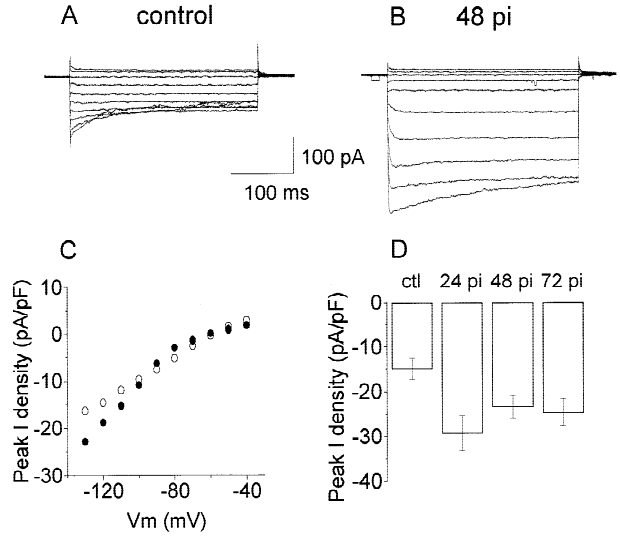

Fig. 3. Infection by *Leishmania amazonensis* increases the density of I_{KIR} in J744.1 cells. (*A*) Membrane currents elicited in a control cell by 300 msec pulses applied from a holding potential of −60 mV to values ranging from −130 to −40 mV, in 10 mV steps every 15 sec. (*B*) Currents recorded 48 hr pi in another cell with the same experimental protocol. The calibration bars in *A* apply also to *B*. (*C*) Relation between the peak current density and the pulse potential, for the cells shown in *A* (empty circles) and *B* (filled circles). The slopes of the *I–V* relations in the −90 to −130 mV range were 0.008 for the control cell and 0.03 for the 48 hr pi cell. (*D*) Pooled data on the density of the peak current elicited by hyperpolarizations to −130 mV from a holding potential of −60 mV. ANOVA tests indicate that the 3 infected groups are significantly different from the control at the *P* value < 0.05 (*n* was: 12, 7, 13, and 7 for control, 24, 48 and 72 hr pi, respectively).

Clapham, 1984; Gallin & Sheehy, 1985, McKinney & Gallin, 1988; McKinney & Gallin, 1992; Randriamampita & Trautman, 1987). Ca^{2+}-activated K^+ channels are also present in these cell types (Gallin, 1984; Randriamampita & Trautmann, 1987). In J774.1 cells, it has been suggested that K^+ inward rectifier channels (KIR) set the cell V_m and that their increased expression is responsible for the hyperpolarization that occurs as a function of time of adherence onto glass (Gallin & Sheehy, 1985; Gallin, 1986). Changes in the properties and/or density of inward rectifying K^+ currents (I_{KIR}) are therefore likely to account for the hyperpolarization we observe after infection. We therefore compared the properties of I_{KIR} in control and infected cells.

I_{KIR} was studied under conditions resembling physiological ones, i.e., low K^+ outside and low Cl^- inside. Furthermore, to limit the washout of KIR described in macrophages and J774 cells (Randriamampita & Trautmann, 1987; McKinney & Gallin, 1988), the internal solution included nucleotides, HEPES buffer and EGTA, as detailed in Materials and Methods. The bath was perfused only when required for addition of pharmacological agents, to avoid the increase in outward current amplitude caused by solution flow (*data not shown;* Randriamampita & Trautmann, 1987). The upper panels of Fig. 3 show records of the currents elicited by 300 msec pulses to potentials ranging from −130 to −40 mV in a control cell (Fig. 3*A*) and in a cell recorded 48 hr pi (Fig. 3*B*). In both cells, the currents had a current-to-voltage (*I–V*) relationship (Fig. 3*C*) and a temporal course consistent with those of KIR. Partial inactivation was evident at potentials negative to −90 mV (Gallin & Sheehy, 1985; McKinney & Gallin, 1988). In control cells, I_{KIR} amplitude increased after 24 hr of adherence onto glass (*data not shown;* McKinney & Gallin, 1990). Therefore, as stated above, time of adherence was kept constant (24 hr) in both control and infected cells. Nonetheless, peak I_{KIR} amplitude was larger in infected cells at potentials

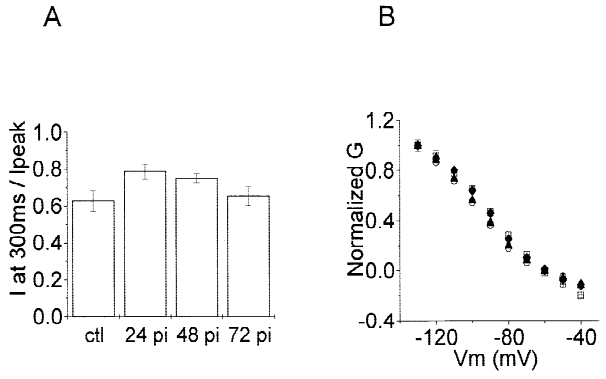

Fig. 4. The kinetic properties and normalized conductance *vs.* V_m curves of I_{KIR} are not affected by *Leishmania amazonensis* infection. (*A*) Pooled data on the ratio of the current amplitude at the end of a 300 msec pulse to −130 mV applied from a holding potential of −60 mV over the peak current amplitude. No significant differences between the control and infected groups were found at the $P < 0.05$ level using ANOVA test. The number of cells for each group was: 12 in control, 7 at 24 pi, 13 at 48 pi and 7 at 72 pi. (*B*) Plot of the normalized conductance (*G*) *vs.* the membrane potential during the pulse for control and experimental groups. Normalized conductance was calculated $G_p/G_{max} = ((A_1 - A_2)/(1 + e^{(V - V_{½})/Sa})) + A_2$, where G_{max} is the maximum value of the conductance, $V_{1/2}$ the potential where *G* has 50% of its peak value and S_a the slope (*see* Table). The graph plots the mean values for each set of data were control (empty squares), 24 hr pi (empty circles), 48 hr pi (filled triangles) and 72 hr pi (filled diamonds, *n* was: 12, 7, 12, and 6 for control, 24, 48 and 72 hr pi, respectively).

below −90 mV (*compare* Fig. 3*A* and *B*). This difference was maintained after normalization by the cell C_m to correct for the increase in surface area induced by infection (Fig. 3*C*).

To quantify the changes in I_{KIR}, we used the peak current density for pulses to −130 mV. Figure 3*D* shows pooled data for this parameter from the 4 experimental groups. By 24 hr pi, I_{KIR} density had increased by 96%, from its control value of −14.9 ± 2.4 pA/pF ($n = 12$) to −29.2 ± 3.8 pA/pF ($n = 7$). For the 48 and 72 hr pi groups, I_{KIR} density remained significantly larger than that of control cells.

We next examined if parameters other than peak current density were altered by infection. The extent of inactivation, measured as the ratio of the current amplitude at the end of a pulse to −130 mV over the peak current did not change with infection (Fig. 4*A*). Furthermore, none of the parameters describing the shape of the normalized *I–V* relation for I_{KIR} (Jerng & Covarrubias, 1997) were altered by infection (*see* Table, *C*). Consequently, the normalized conductance (*G*) *vs.* V_m curves, where *G* is divided by G_{max} to correct for the changes in current density observed, superimposed for all the experimental groups, as shown in Fig. 4*B*.

KIR channels in macrophages and J774.1 cells have low sensitivity to extracellular tetraethylammonium (TEA) but are inhibited by external Ba^{2+} (reviewed by Gallin, 1986). Infected cells had a similar pharmacological profile, regardless of the time pi. In all cells studied, TEA (5 to 10 mM) had little effect on I_{KIR}, whereas external Ba^{2+} strongly reduced current amplitude. Figure 5 shows the currents recorded from a 24 hr pi cell in control external saline (*A*), in the presence of 10 mM TEA (*B*) and after addition of 3 mM Ba^{2+} (*C*). In 9 cells tested, TEA had a minor effect on I_{KIR}, decreasing the peak current at −130 mV by only 12.7 ± 2.6%. Subsequent addition of 3 mM Ba^{2+} to 4 of these cells reduced the peak current by 78.2 ± 6.5%. In 5 other cells tested only with 3 mM Ba^{2+}, the block was 74.5 ± 4.4%.

Given the large fraction of I_{KIR} blocked by Ba^{2+}, we tested whether this channel blocker could reverse the hyperpolarization observed in infected macrophages. In 5 cells studied at 72 hr pi, addition of 3 mM Ba^{2+} to the bath solution resulted in a shift in V_m from −62 ± 1.4 mV to −50 ± 1.3 mV, a value not significantly different from the V_m of control cells. These results indicate that the pharmacological properties of I_{KIR} are not affected by *L. amazonensis* infection and that the changes in V_m are associated with a Ba^{2+}-sensitive current.

Discussion

Leishmania parasites have adapted to living in the hostile lysosomal-like environment of the PV formed within macrophages. Despite work on other closely related protozoa like *T. cruzi*, *Toxoplasma gondii* and *Plasmodium* spp, an understanding of how these parasites affect the host cell membrane and how specific these changes might be, remains fragmentary. In this study, we show that infection by *L. amazonensis* leads to changes in basic electrical properties and in one of the ionic currents of J774.1 cells.

Possible Mechanisms of Membrane Surface Increase

The two basic electrical parameters altered by infection are the cell membrane capacitance and their resting membrane potential. It is interesting to note that the ratio of C_m/D_m increased during infection, suggesting a change in the extent of membrane folding. However, the observed increase in C_m is most likely to be due to incorporation of new membrane to the cell plasma membrane. The question then arises as to whether this membrane is produced *de novo* by the host cell, is the result of a change in membrane recycling, or is donated by the parasite.

In erythrocyte infection by *Plasmodium*, the parasite donates membrane to the PV. However, there does not appear to exist strong evidence of the same phenomenon towards the surface membrane of the host cell (reviewed

Table.

	Control	24 pi	48 pi	72 pi
A				
A_1	1.12 ± 0.03	1.14 ± 0.35	1.25 ± 0.06	1.09 ± 0.02
A_2	−0.33 ± 0.02	−0.12 ± 0.01	−0.19 ± 0.02	−0.16 ± 0.01
$V_{1/2}$	−87.89 ± 0.69	−97.54 ± 0.99	−99.13 ± 1.63	−90.93 ± 0.64
S_a (*e*-fold)	21.11 ± 0.99	16.28 ± 0.96	19.85 ± 1.57	16.28 ± 0.74
B				
*s*1	−0.013 ± 0.001	−0.015 ± 0.002	−0.016 ± 0.002	−0.014 ± 0.001
r	−0.994	−0.996	−0.997	−0.991
C				
*s*2	0.016 ± 0.001	0.017 ± 0.002	0.018 ± 0.002	0.016 ± 0.001
r	0.999	0.999	0.999	0.991
n	11	7	12	6

(A) Activation parameters. Peak conductance (G_p) was calculated as $G_p = I_p/(V - V_r)$, where I_p is the peak current, V the membrane potential during the pulse and V_r the K^+ reversal potential predicted by the Nernst equation (−84 mV). The normalized $G-V$ curve was well described by a first order Boltzmann function of the form $G_p/G_{max} = ((A_1 - A_2)/(1 + e^{(V-V_{1/2})/Sa})) + A_2$, where G_{max} is the maximum value of the conductance, $V_{1/2}$ the potential where G has 50% of its peak value and S_a the slope. (B) Parameters obtained from the fit of the normalized conductance-voltage ($G-V$) relation between −90 and −130 mV to a straight line, where *s*1 gives the slope and r the correlation coefficient. (C) Parameters describing the normalized current-voltage ($I-V$) relation between −90 and −130 mV, where *s*2 gives the slope and *r* the correlation coefficient. The last row gives the number of cells (*n*) for each experimental group. ANOVA and Dunnett tests indicated that none of the fit parameters presented in this table differed significantly between the experimental groups at the $P < 0.05$ level.

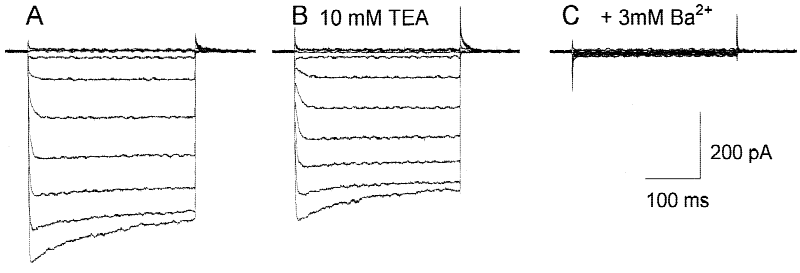

Fig. 5. Pharmacological profile of I_{KIR} in infected J774.1. (*A*) Membrane currents elicited by an identical protocol to that described for Fig. 3*A*, recorded at 24 hr pi in control external solution. (*B*) Currents obtained in the same cell, with the same experimental protocol, after addition of 10 mM TEA to the bath solution. (*C*) Records from the same cell after addition of 3 mM Ba^{2+} to the TEA-containing external solution. The calibration bars in *C* apply to the 3 panels.

by Elmendorf & Haldar, 1993). In peritoneal macrophages infected by *L. amazonensis*, there is evidence for lysosomal depletion (Barbieri, Brown & Rabinovitch, 1985). This could result from lysosomal fusion and decreased vesicle budding from the PV, and may reflect a more general phenomenon of altered membrane recycling in macrophages. It is also known that macrophages continuously replace their surface membrane and infection may stimulate its synthesis and incorporation.

Hyperpolarization and its Physiological Significance

In the present work, J774.1 cells hyperpolarized after *L. amazonensis* infection. This increase in V_m was not a consequence of time of adherence onto glass (Gallin & Sheehy, 1985) since this parameter was kept constant in all experimental groups. Several other mechanisms have been reported to produce changes in macrophage V_m, including activation for the Na^+/Ca^{2+} exchanger (Donnadieu & Trautmann, 1993), gating by glycine of Cl^- channels (Ikejima et al., 1997) and modifications of K^+ channels (*see below*). The presence of EGTA and low Cl^- in our intracellular solutions makes it unlikely that the Na^+/Ca^{2+} exchanger or Cl^- channels account for the hyperpolarization observed.

Modifications of K^+ currents most probably explain the sustained hyperpolarization elicited by infection. Murine macrophages and L fibroblasts undergo sustained hyperpolarization after phagocytosis. In macrophages, KIR channels are considered to set the membrane potential and the hyperpolarization induced by phagocytosis has been attributed to changes in I_{KIR} (Kouri, 1980; Gallin, 1986). In fibroblasts, the change in V_m is necessary for microparticle ingestion and is Ca^{2+} dependent, since

removal of external Ca^{2+} or block of Ca^{2+} channels inhibits both, phagocytosis and hyperpolarization (Okada et al., 1981). Extracellular Ba^{2+} diminished the uptake of beads in L fibroblasts (Okada et al., 1981) suggesting a functional relation between I_{KIR} and phagocytosis. Other phagocytic cells such as human neutrophils, respond to chemotactic stimulus with an early and transient hyperpolarization, a large and transient increase in cytosolic Ca^{2+}, increased K^+ permeability and intracellular acidification (Lazzari, Proto & Simons, 1990). In monocytes, *Leishmania* infection is associated with an increase in basal Ca_i levels and alterations on Ca^{2+}-dependent pathways (reviewed by Oliver, 1996) which may change the functional expression of KIR channels, as discussed below.

Association Between I_{KIR} and Hyperpolarization in *Leishmania* Infected J774.1 Cells

The hyperpolarization observed after infection was sustained for as long as 72 hr pi and it was paralleled by an increased in the density of I_{KIR}. Furthermore, extracellular Ba^{2+}, which blocked a large fraction of I_{KIR} in all experimental groups, reversed the hyperpolarization in infected cells. Taken together, these data indicate a link between hyperpolarization and increased I_{KIR} density. The characteristics of I_{KIR} we report are similar to those of the KIR2.1 channels cloned from J774 cells (Kubo et al., 1993; Doupnik, Davidson & Lester, 1995; Redell & Tempel 1998). This clone has also been referred as IRK1 and Kcnj2. When studied in heterologous expression systems, the channels codified by this gene have strong rectification, are blocked by extracellular Ba^{2+}, tend to saturate at high hyperpolarizing potentials, have a rather slow inactivation, a linear *I–V* relationship between −130 and −100 mV, and activate at around −80 mV in 5 mM extracellular K^+ (Kubo et al., 1993; Ruppersberg & Fakler, 1996). All these features are consistent with a predominant KIR2.1-like current in J774.1 cells.

The increase in I_{KIR} current density in *L. amazonensis* infected J774.1 cells was not accompanied by changes in the temporal course, in the shape of the *I–V* and *G–V* relations and in the pharmacological profile of this current. Therefore, the hyperpolarization cannot be explained by the appearance of different type(s) of channels, nor by a shift in the activation voltage of existing ones. It could arise either from a change in the single channel conductance or from an increase in the number of functional channels. KIR2.1 channels are modulated post-translationally by many factors (Taglialatela et al., 1994; Ruppersberg & Fakler, 1996; Shieh et al., 1996; Lopatin & Nichols, 1996; Huang, Feng & Hilgemann, 1998). However, post-translational modifications are unlikely to explain the long-term effects observed in V_m and I_{KIR} current density of infected J774.1 cells. Transcriptional modulation may do. KIR2.1 mRNA levels increase in muscle cells with elevated Ca_i (Shin et al., 1997) and in human monocytic leukemia cells induced to differentiate through activation of protein kinase C (PKC) by phorbol esters (DeCoursey et al., 1996). Therefore, it is plausible that the changes produced by *Leishmania* infection in the basal Ca_i levels (reviewed by Oliver, 1996) and PKC activity (Descorteaux, Matlashewski & Turco, 1992) of its host cell lead to an increase in the density of KIR channels. However, analysis of KIR channels in control and infected cells at the single channel level will be required to establish whether changes other than the number of functional channels, i.e., single channel conductance, are altered by infection.

In summary, hyperpolarization appears to be associated with activation and phagocytosis in macrophages. Present information does not allow a solid conclusion regarding the physiological role of the activity-dependent hyperpolarization found in different phagocytic cells. Whether hyperpolarization is important for parasite invasion and/or survival is a question that requires further investigation.

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