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McKinney, L.C., and Gallin, E.K.

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Membrane Biology

Effect of Adherence, Cell Morphology, and Lipopolysaccharide on Potassium Conductance and Passive Membrane Properties of Murine Macrophage J774.1 Cells

Leslie C. McKinney and Elaine K. Gallin
Department of Physiology, Armed Forces Radiobiology Research Institute, Bethesda, Maryland 20814

Summary. The effects of adherence, cell morphology, and lipopolysaccharide on electrical membrane properties and on the expression of the inwardly rectifying K conductance in J774.1 cells were investigated. Whole-cell inwardly rectifying K currents (K_i), membrane capacitance (C_m), and membrane potential (V_m) were measured using the patch-clamp technique. Specific K_i conductance (G_K, whole-cell K_i conductance corrected for leak and normalized to membrane capacitance) was measured as a function of time after adherence, and was found to increase almost twofold one day after plating. Membrane potential (V_m) also increased from -42 ± 4 mV ($n = 32$) to -58 ± 2 mV ($n = 47$) over the same time period. G_K and V_m were correlated with each other; G_l (leak conductance normalized to membrane capacitance) and V_m were not. The magnitudes of G_K and V_m 15 min to 2 hr after adherence were unaffected by the presence of 100 μM cycloheximide, but the increase in G_K and V_m that normally occurred between 2 and 8 hr after adherence was abolished by cycloheximide treatment. Membrane properties were analyzed as a function of cell morphology, by dividing cells into three categories ranging from small round cells to large, extremely spread cells. The capacitance of spread cells increased more than twofold within one day after adherence, which indicates that spread cells inserted new membrane. Spread cells had more negative resting membrane potentials than round cells, but G_K and G_l were not significantly different. Lipopolysaccharide (LPS; 1 or 10 μg ml) treated cells showed increased C_m compared to control cells plated for comparable times. In contrast to the effect of adherence, LPS-treated cells exhibited a significantly lower G_K than control cells, indicating that the additional membrane did not have as high a density of functional G_K channels. We conclude that both adherence and LPS treatment increase the total surface membrane area of J774 cells and change the density of K_i channels. In addition, this study demonstrates that membrane area and density of K_i channels can vary independently of one another.

Key Words potassium channel · macrophage · J774.1 cells · lipopolysaccharide · adherence · ion transport

Introduction

A number of different K currents have been described in macrophages or macrophage-like cell lines (for review *see* Gallin & McKinney, 1989). Several of these studies have shown that ionic currents change with the functional state of the cell. In human peripheral blood monocytes, large conductance Ca-activated K channels appear in the plasma membrane over a time course of 4–5 days as the cells mature from monocytes into macrophages (Gallin & McKinney, 1988), while different, smaller conductance Ca-activated K channels are present both shortly after isolation and after 4–5 days in culture (Gallin, 1989). In addition, exposure of human monocytes to lipopolysaccharide (LPS), an 'activating' or 'priming' stimulus, increases the percentage of cells expressing a transient outward K current (K_t) from 0 to approximately 30% (Jow & Nelson, 1989). In the murine macrophage-like cell line J774.1, a K_t current is briefly expressed immediately after cells become adherent, while an inwardly rectifying K current (K_i) increases in magnitude over a time course of approximately one day (Gallin & Sheehy, 1985). Adherent mouse peritoneal macrophages also express a K_i current after five days in culture (Randriamampita & Trautmann, 1987), but do not appear to express this conductance before that time (Ypey & Clapham, 1984).

These studies demonstrate that the state of maturation of the macrophage, as well as adherent culture conditions or exposure to LPS, can affect the expression of K channels in macrophages. It is well known that adherence induces functional changes in macrophages that are associated with cell activation. (For a general discussion of macrophage activation *see* Cohn (1978) or Karnovsky and Lazdins (1978).) These include increased synthesis or secretion of various lymphokines (Fuhlbrigge et al., 1987; Haskill et al., 1988), and an increase in the oxidative burst induced by phorbol myristate acetate (PMA; Berton & Gordon, 1983; Kunkel & Duque, 1983). Other adherence-induced changes include an increase in resting membrane potential (Sung et al., 1985), increased amino acid transport

(Pofit & Strauss, 1977), transiently increased levels of IP_3 (Zabrenetzky & Gallin, 1988), development of peroxidase activity within 2 hr post-adherence (Bodel, Nichols, & Bainton, 1977), and expression of several surface antigens that are not expressed in cells in suspension (Triglia, Burns & Werkmeister, 1985; Midoux et al., 1989).

Exposure to LPS also induces a variety of changes in macrophages and macrophage cell lines, including increased spreading (Pabst & Johnston, 1980) and membrane ruffling of adherent cells (Morland & Kaplan, 1977), and stimulation of lymphokine synthesis and secretion (Zacharchuk et al., 1983; Fuhlbrigge et al., 1987). Most importantly, LPS 'primes' the cell to respond to other stimuli (Gordon, Unkeless & Cohn, 1974; Pabst, Hedegaard & Johnston, 1982). In J774.1 cells, LPS inhibits cell division (Ralph & Nakoinz, 1977; Kurland & Bockman, 1978; Okada et al., 1978) and induces secretion of prostaglandin E (Kurland & Bockman, 1978) and T-cell activating factors (Okada et al., 1978).

The purpose of this study was to determine the effects of both adherence and LPS on the expression of the inwardly rectifying K conductance and on the electrical membrane properties (membrane potential, V_m; leak resistance, R_L; and capacitance, C_m) of the murine macrophage-like cell line J774.1. While Gallin and Sheehy (1985) noted that the magnitude of K_i in J774.1 cells increased with time after adherence, they did not determine whether the increased current magnitude was due to an increase in cell size, or whether current density (current/unit membrane area) increased. This question was resolved in the present study by directly measuring whole-cell inwardly rectifying K conductance and normalizing this value to C_m to obtain specific K_i conductance (G_{K_i}). Since membrane capacitance is directly proportional to membrane area, these measurements also yielded information about the effect of adherence and LPS on the insertion of new membrane by the cell. We also examined the effect of cycloheximide, a protein synthesis inhibitor, on the expression of G_{K_i} following adherence, since protein synthesis inhibitors have been shown to interfere with the expression of other surface antigens in the macrophage (Smith & Ault, 1981; Triglia et al., 1985). Finally, the expression of G_{K_i} as a function of cell shape was characterized, to determine if G_{K_i} was differentially expressed in very spread *versus* nonspread cells.

Materials and Methods

Cell Culture

J774.1 (J774A.1) cells were obtained from American Type Tissue Culture (Rockville, MD) and maintained in suspension at 37°C for not more than 40 days. Cells were fed at least 12 hr prior to plating for electrophysiological experiments. J774.1 cells have a doubling time of <24 hr and were not synchronized with respect to cell cycle. Whittaker RPMI 1640 culture medium (Bioproducts, Walkersville, MD) was supplemented with 5% fetal calf serum, 4 mM glutamine, and 100 U/ml penicillin-streptomycin (DIFCO). For recording, cells were placed on glass coverslips and maintained in culture medium for varying times (minutes to days). Prior to patch-clamp recordings, coverslips were mounted in a plexiglass chamber in 300–400 μl of Na Hanks', maintained at room temperature (23 to 26°C), and the recording media changed every 20 to 30 min. Coverslips were recorded from for no more than 1 hr.

Recording Methods

Recording methods were the same as those previously described (McKinney & Gallin, 1988). Briefly, whole-cell current records were obtained using a List (Darmstadt, FRG) EPC-7 patch clamp. Voltage pulses were generated by computer and currents were digitized, displayed, and analyzed using an Indec Laboratory Display System (Sunnyvale, CA). Patch electrodes of 3–5 MΩ resistance were made from hematocrit glass (#02-668-68, Fisher Scientific, Pittsburgh, PA). Zero current potentials were measured in current clamp mode immediately after attainment of the whole-cell configuration, and cells were held within 5 mV of this value.

Analytical Methods

Whole-cell records were corrected for leak and capacity currents. Electrode capacitance was compensated while in the cell-attached mode. Total membrane capacitance (C_m) was measured in the whole-cell mode by integrating the capacity transient and was then compensated electronically. Series resistance (R_s) was measured either directly from the EPC-7 after capacity transient cancellation or by fitting a capacitance record with a mono-exponential function to find τ and calculating R_s from the relationship $R_s = \tau/C$. The average series resistance for 95 cells was 6.9 ± 0.6 MΩ. Leak current was measured in one of two ways: by measuring the current in the voltage range −45 to −32 mV where no time-dependent current was present, or by measuring the current at negative voltages in the presence of 2 mM barium, which blocks all inwardly rectifying current and results in a linear I–V relationship. Leak resistances (R_L) obtained using either method were not significantly different from one another: R_L = 2.7 ± 0.3 GΩ (n = 89) in the absence of barium and R_L = 3.0 ± 0.3 GΩ (n = 39) in the presence of barium. For purposes of comparison to inwardly rectifying K conductance measurements, leak resistance values were converted to units of conductance [R_L (GΩ) = 1/G (nS)]. Specific leak conductance (G_L) was determined by dividing the leak conductance by total membrane capacitance. As in our previous study (McKinney & Gallin, 1988), for calculations of channel density (# channels/μm² of membrane), the specific capacitance of the cell membrane was assumed to be 1 μF/cm². Values of surface area calculated using this value compare well with values reported for mouse peritoneal macrophages obtained by stereologic analysis (Steinman, Brodie & Cohn, 1976). Values of surface area obtained using either capacitance measurements or stereologic techniques are two- to threefold larger than surface area measurements calculated from values of cell diameter (assuming a spherical cell shape). This is because macrophages are irregularly shaped and can have considerable membrane ruffling and infolding.

Whole-cell conductance for inward current (K_i) was measured in the following way. Peak current amplitude was measured at the beginning of a hyperpolarizing voltage step by eye using a cursor. Current vs. voltage (I–V) curves were constructed and a straight line fitted to the data for steps between −90 to −160 mV to yield a conductance value. After subtraction of leak conductance, values were normalized to membrane capacitance to yield a value for specific K conductance G_K.

In order to get an accurate sampling of whole-cell conductance values from the population of J774.1 cells, cells were not excluded on the basis of low resting membrane potentials or small inward current amplitudes. After verifying increased capacitance after rupture of the patch (the lowest value accepted was 11 pF) and an acceptable series resistance value (not greater than 20 MΩ) for a particular recording, we included virtually all cells that had stable membrane properties in the study.

^{3}H-Leucine Incorporation

The effect of cycloheximide on protein synthesis was assayed by measuring incorporation of ^{3}H-leucine into protein in the presence of varying concentrations of the protein synthesis inhibitor cycloheximide (Sigma Chemical, St. Louis, MO). An IC_{50} of approximately 1 μM was observed; 100 μM cycloheximide inhibited greater than 90% of ^{3}H-leucine incorporation in these cells.

Solutions

Cells were bathed in a Na Hanks' solution consisting of (in mM): 145 NaCl, 4.5 KCl, 1.6 $CaCl_2$, 1.1 $MgCl_2$, 10 HEPES NaOH buffer, pH 7.3. The pipette contained (in mM): 145 KCl, 1 $MgCl_2$, 1.1 EGTA, 0.1 $CaCl_2$, and 10 HEPES KOH, pH 7.3. Free Ca in this solution was less than 10^{-8} M. *Escherichia coli* derived lipopolysaccharide (LPS) was obtained from DIFCO Laboratories (Detroit, MI).

Statistical Methods

Unless otherwise stated, mean values were considered to be significantly different from one another if $P < 0.05$ using a Student's t test. Linear regression analysis was carried out on a VAX 11 750 using the RSI statistical package (BBN Software Products, Cambridge, MA). Two values were said to be correlated if the R value of the slope of the line relating the two variables was significantly different from zero ($P < 0.05$). In some cases, in order to analyze upward or downward trends in the data with time, biweight regressions were done and slopes were compared to zero. Slopes of control vs. test data were compared to each other using a t test.

Results

Membrane Capacitance, Specific K_i Conductance, and Membrane Potential Increase with Time After Adherence

Figure 1*A* shows an example of inwardly rectifying K_i currents recorded from J774.1 cells bathed in Na Hanks, with the corresponding I–V relationship shown in Fig. 1*B*. The K_i current activates at −50 mV, and shows time-dependent inactivation below about −120 mV that is partially due to block by Na, and partially due to inactivation of the current (McKinney & Gallin, 1988). Properties of both the whole-cell current and the single-channel events underlying them have been described in detail elsewhere (McKinney & Gallin, 1988). The K_i current is completely blocked by 1 mM barium and in >95% of the recordings was the only time-dependent current observable under these recording conditions. Therefore, the leak-subtracted whole-cell conductance measured over negative voltages appears to be due solely to the conductance of the K_i current. An inactivating outward K current (K_o) was observed in about 5% of cells, but it activated at potentials above −40 mV (Gallin & Sheehy, 1985), and did not interfere with measurements of the inwardly rectifying current. Randriamampita and Trautmann (1987) have reported the existence of a linear Ca-dependent K current in J774 cells which is apparent only when intracellular calcium is buffered at 1 μM. In our experiments, $[Ca]_i$ was buffered at 10^{-8} M and so this current was not observed.

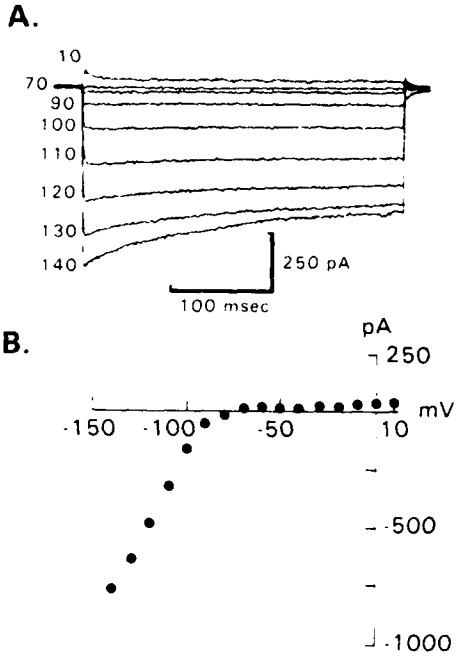

Fig. 1. (*A*) Inwardly rectifying K current. Cell was held at −70 mV and 440 msec voltage pulses applied in 10-mV increments. Records have not been leak subtracted to show total current. (*B*) Peak current-voltage plot for experiment shown in *A*

In order to study how the K_i conductance and other membrane properties change after adherence, whole-cell recordings were obtained at varying times after plating. Values for C_m, G_K, V_m, and G_l are plotted in the form of bar graphs showing the mean ± SEM as a function of time after adherence

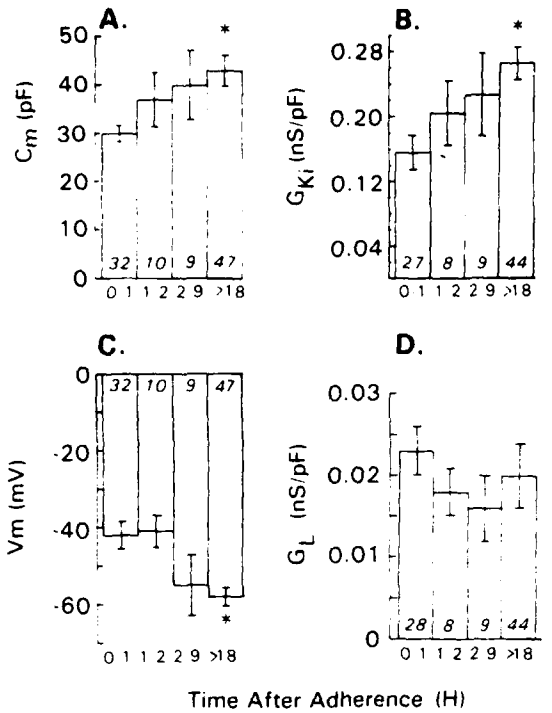

Fig. 2. Plots of (*A*) C_m (*B*) G_{K_i}, (*C*) V_m, and (*D*) G_l *vs.* time after adherence. Values plotted are means ± SEM. Asterisk (*) indicates values which are significantly different from values at 0–1 hr. Numbers within the bar, for these and all subsequent graphs, indicate number of cells studied

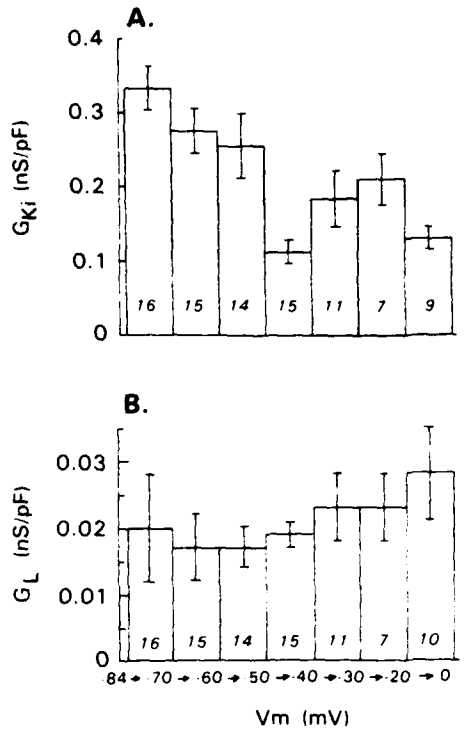

Fig. 3. (*A*) Plot of G_{K_i} *vs.* resting V_m. (*B*) Plot of G_l *vs.* V_m. Conductance values given are mean ± SEM. V_m values (in mV) were grouped as follows: −84 to −70, −69 to −60, −59 to −50, −49 to −40, −39 to −30, −29 to −20, −19 to 0

(Fig. 2). Cells were grouped into four time periods: 0–1 hr (to follow changes in membrane properties at early times after adherence), 1–2, 2–9, and >18 hr. It should be noted that the earliest time point at which recordings were made was approximately 15 min after adherence, and no recordings were made at times longer than about 36 hr after adherence.

Significant changes in membrane properties were noted following adherence. Membrane capacitance increased significantly with time after plating (Fig. 2*A*) from 30 ± 2 pF to 43 ± 3 pF. Assuming that 1 cm^2 of membrane has a capacitance of 1 μF, the average membrane area of the cells increased by about 40%. Thus, the morphological changes which J774.1 cells undergo after adherence do not merely represent shape changes but reflect a net insertion of additional membrane. G_{K_i} increased almost twofold over 18 hr (Fig. 2*B*), from 0.16 ± 0.02 nS/pF to 0.27 ± 0.02 nS/pF. Since this value is normalized to membrane area, the increase in G_{K_i} was not due to the increased membrane area of the cells. Our single-channel data indicate that the conductance of single inwardly rectifying channels does not change after adherence (*data not shown*). Therefore, the increase in G_{K_i} represents an increase in the density of K_i channels in the membrane over this time period from 44 channels/pF (0.44 channels/μm^2) to 75 channels/pF (0.75 channels/μm^2). These data were supported by direct observations of K_i channel activity in cell-attached patches (McKinney & Gallin, 1988). Membrane patches from freshly plated cells usually contained few (0–3) channels, while membrane patches from cells adherent for one day usually contained many (2–6) channels. Since both channel density and membrane area increased with time after adherence, the average number of channels per cell increased from 1,320 to 3,225 over 18 hr.

During the 18-hr period following adherence, the average V_m of the cells increased from −42 ± 4 mV to −58 ± 2 mV (Fig. 2*C*). The increase in membrane potential could be due to an increase in G_K or could also be due to a concomitant decrease in G_L. However, as shown in Fig. 2*D*, G_L did not change significantly with time after adherence, indicating that the cells maintained a rather constant leak conductance over the time that C_m, V_m, and G_{K_i} were increasing.

Specific K_i Conductance and Membrane Potential Are Correlated

Figure 3 shows the relationship between G_{K_i} and resting V_m. (Note: resting V_m is actually determined

Table 1. Effect of cycloheximide on membrane properties of J774.1 cells

	Time after adherence (hr)	G_K (nS/pF)	G_l (nS/pF)	V_m (mV)	C_m (pF)
Control	0–2	0.16 ± 0.02 (35)	0.02 ± 0.003 (36)	−42 ± 3 (42)	31 ± 2 (42)
Cycloheximide-treated	0–2	0.25 ± 0.04 (7)	0.03 ± 0.006 (8)	−51 ± 6 (10)	35 ± 4 (10)
Control	2–9	0.23 ± 0.05 (9)	0.02 ± 0.004 (9)	−55 ± 8 (9)	40 ± 7 (9)
Cycloheximide-treated	2–8	0.14 ± 0.04 (14)	0.03 ± 0.006 (15)	−54 ± 5 (15)	30 ± 2 (15)

Note that cells were exposed to cycloheximide for 1 hr prior to plating, as well as during plating.

by steady state, not peak K conductance. However, over the voltage range of −50 to −110 mV, peak and steady-state conductance are equivalent.) Data are from cells plated at all times. Between −84 and −40 mV there was a positive correlation between G_K and resting V_m. That is, over the range at which the inwardly rectifying K conductance is activated, cells which had higher conductance to K_i also had a more negative resting membrane potential. In contrast, there was no correlation between G_K and V_m for voltages positive to −40 mV. In addition, there was no correlation between V_m and G_l over any voltage range. This result indicates that cells which had low resting membrane potentials were not depolarized merely because they were 'leakier'. Likewise, there was no correlation between G_K and G_L: cells with a low specific K_i conductance were not necessarily those with a large leak conductance. Thus, it is likely that the increase in resting V_m observed over the first 24 hr of adherence is a function of the increased conductance to K_i, and not a function of a change in G_L.

Initial Expression of G_K Is Not Inhibited by Cycloheximide

To determine (*i*) whether the observed increase in the density of K_i channels after adherence depended on protein synthesis and (*ii*) whether inhibition of protein synthesis before cells were allowed to become adherent would interrupt the initial expression of K_i, G_K was measured in cells treated with the protein synthesis inhibitor cycloheximide, which was shown to inhibit ^{3}H-leucine incorporation in these cells (*see* Materials and Methods). Cells were exposed to cycloheximide (100 μM) for 1 hr prior to plating, plated in the presence of cycloheximide, and G_K was measured at various times after plating. Cells treated with cycloheximide were able to adhere and spread similar to untreated cells, and the general morphology appeared normal. However, Gigaohm seals were considerably less stable, which made recording from the cells for an extended period of time difficult. Values for G_K, G_L, V_m and C_m are given in Table 1, grouped into two time periods: 0–2 and 2–8 hr after adherence. Because prolonged incubation in cycloheximide may affect cell viability, the conductance of cells which had been plated in cycloheximide for more than 8 hr was not measured. Cycloheximide did not inhibit the initial expression of inward current. Average G_K 0–2 hr after adherence was not significantly different from the average G_K value for untreated cells plated for the same amount of time. Values for G_L, V_m and C_m were also not different from controls. However, with time after adherence, cycloheximide did inhibit the increase in G_K and V_m normally observed in control cells. While control cells increased their mean G_K from 0.16 to 0.23 nS/pF 2–9 hr after adherence, the mean G_K of cycloheximide-treated cells decreased from 0.25 to 0.14 nS/pF. When G_K values were plotted *vs.* time for cycloheximide-treated cells, the slope of the line was found to decline significantly (Fig. 4). Cycloheximide-treated cells did not show a significant trend to more negative resting membrane potentials with time after adherence, nor was there a significant trend toward increased membrane capacitance as was shown by control cells. G_L of cycloheximide-treated cells was unchanged after adherence. These data indicate that cycloheximide does not interfere with the initial expression of the inwardly rectifying K conductance, but does inhibit the upregulation of K channels and the insertion of new membrane which occurs following adherence.

Different Morphological Groups Have Different Membrane Properties

Adherent J774.1 cells exist in a variety of shapes and sizes that may reflect different functional states. For instance, cells undergoing cell division become rounded while migrating cells have a polarized appearance, with a leading and trailing edge. Other cells exhibit a very flattened appearance almost immediately after adherence. In order to de-

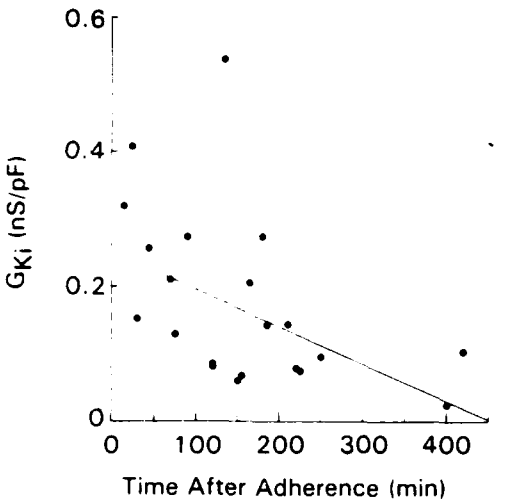

Fig. 4. Plot of G_K vs. time after adherence for cycloheximide-treated cells. Solid line is a linear biweight fit to the data. Its slope is significantly different from zero. Note that cells were exposed to cycloheximide 1 hr prior to plating, as well as during plating

termine whether cells of different morphologies had different membrane properties, cells were divided into three groups according to shape (*see* photograph, Fig. 5): group *#1*, small round cells without visible processes, mean capacitance 27 ± 3 pF (n = 20), average diameter 22 μm; group *#2*: somewhat spread or polarized cells, some with visible processes, mean capacitance 36 ± 2 pF (n = 40), average dimensions 37 × 28 μm; and group *#3*: extremely spread cells, mean capacitance 45 ± 4 pF (n = 36), average dimensions 50 × 42 μM. V_m, C_m, G_K, and G_L for each morphological category were determined for two time periods: 0–2 and >18 hr after adherence (Fig. 6). Examining the data in this way revealed some interesting differences between cell types that were not apparent when the data from all cells were pooled (as in Fig. 2).

Values for V_m, C_m, G_K and G_L were not significantly different between groups *1* and *2* at either 0–2 hr or >18 after adherence. Thus, for clarity, only data from groups *1* and *3* are shown in Fig. 6. Very spread cells were different from round cells in several respects. They were significantly more hyperpolarized than round cells at either 0–2 or > 18 hr following adherence (Fig. 6*A*). Also, one day after adherence, spread cells had much larger membrane capacitance than round cells (Fig. 6*B*). Both round and spread cells still showed a positive correlation between V_m and G_K. However, G_K (Fig. 6*C*) and G_L (Fig. 6*D*) were not significantly different between groups *1* and *3* compared at the same time period (0–2 or >18 hr).

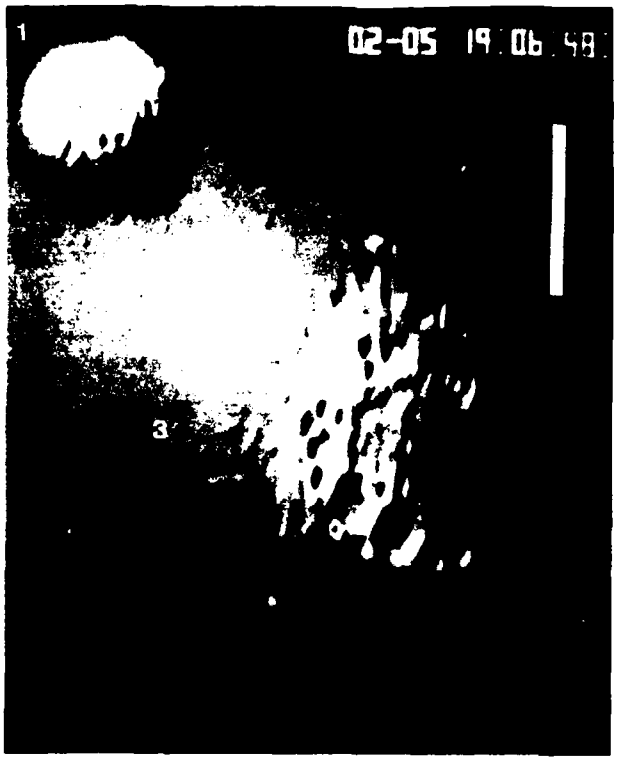

Fig. 5. Photograph of adherent J774.1 cells showing different morphological categories. Bar: 20 μm

We also examined how membrane properties changed with time after adherence for each group. First, only the very spread cells showed a significant (almost twofold) increase in membrane capacitance over 18 hr (Fig. 6*B*). Thus, most of the previously observed increase in membrane area which occurs after adherence (*see* Fig. 2*A*) can be attributed to the increased membrane area of very spread cells. Second, membrane potential significantly increased with time after adherence for both groups of cells (Fig. 6*A*). Round cells showed the largest increase in V_m, and also showed the largest increase in G_K, 18 hr after adherence (Fig. 6*C*). Spread cells showed a smaller increase in V_m, and a correspondingly smaller increase in G_K. G_L values did not change after adherence for either round or spread cells (Fig. 6*D*). We conclude that very spread cells behave differently from round cells after adherence: they insert more membrane but do not significantly increase K_t current density after adherence.

LPS-Treated Cells have Different Membrane Properties

The effect of LPS on membrane properties of J774.1 cells was examined by incubating cells in suspen-

Table 2. Effect of LPS on membrane properties of J774.1 cells

	Time after adherence (hr)	G_K (nS pF)	G_L (nS pF)	V_m (mV)	C_m (pF)
Control	0–2	0.16 ± 0.02 (35)	0.02 ± 0.003 (36)	−42 ± 3 (42)	31 ± 2 (42)
LPS-treated	0–2	0.09 ± 0.02 (13)	0.02 ± 0.007 (14)	−37 ± 5 (16)	42 ± 5 (17)
Control	>18	[b]0.27 ± 0.02 (44)	0.02 ± 0.004 (44)	[b]−58 ± 2 (47)	[b]43 ± 3 (47)
LPS-treated	>18	[a,b]0.15 ± 0.02 (8)	0.02 ± 0.006 (9)	[b]−62 ± 4 (10)	[a,b]68 ± 10 (10)

[a] Significantly different from control cells at the same time period.
[b] Significantly different from the value at 0–2 hr.
Note that cells were exposed to LPS one day prior to plating, as well as during plating.

sion with 1 or 10 μg/ml of LPS overnight and then plating cells for various times in the presence of LPS. Values for G_K, G_L, V_m and capacitance are given in Table 2, grouped into two time periods, 0–2 and 18 hr after adherence. Data were not separated into different morphological categories; LPS-treated cells were of all three groups but primarily of group #2. For a given time period after adherence, V_m and G_L values were comparable to those of control cells, but G_K values were significantly lower. However, G_K and V_m were still correlated; G_L and V_m were not. Membrane capacitance was larger, indicating that LPS-treated cells have a larger membrane surface area. Thus, activation of cells by LPS resulted in the insertion of new membrane, but did not result in increased density of K channels. Membrane properties of LPS-treated cells showed changes with time after adherence in a manner similar to control cells. G_K, V_m and C_m all increased significantly one day after adherence, as they did for untreated cells, while G_L was unchanged.

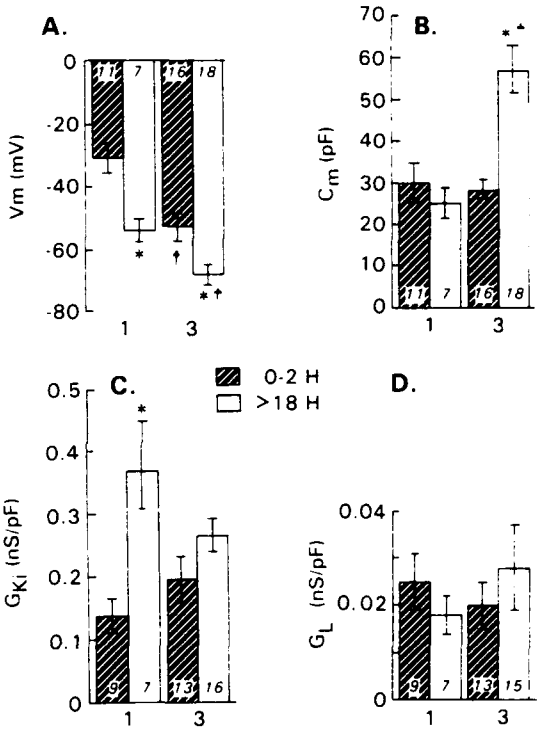

Fig. 6. (*A*) V_m, (*B*) C_m, (*C*) G_{K_i}, and (*D*) G_L with time after adherence for round (group *1*) and spread (group 3) cells. Mean values were compared at 0–2 and >18 hr for each group. *Indicates values that are significantly different from values from the same group at 0–2 hr. †Indicates values that are significantly different from values of group *1* at the same time period

Discussion

This study demonstrates that membrane properties of J774. cells vary with time after adherence, cell morphology, and after exposure to LPS. Values for membrane capacitance (C_m), membrane potential (V_m), and specific K conductance (G_K) were significantly increased 18 hr after adherence. As shown in Fig. 1, these trends were evident at times earlier than 18 hr post-adherence, even though statistically significant differences between mean values were not yet demonstrable. As a point of comparison, previous studies in T cells demonstrated that stimulation with the mitogens phorbol myristate acetate or concanavalin A caused an increase in K channel density over a time course of about one day (Deutsch, Krause & Lee, 1986; DeCoursey et al., 1987). The increase in channel density occurred over the same time course as the increase in protein synthesis induced by mitogen.

The observed increase in membrane capacitance after plating provides the first evidence that J774.1 cells actively increase their membrane area following adherence. We found that very spread cells showed the largest increase in membrane area following adherence (>twofold), while small round

cells showed very little change. This does not imply that the membrane of smaller cells is unchanging, however. It has been shown that mouse peritoneal macrophages can pinocytose their plasma membrane at high rates (3.1%/min; Steinman, Brodie & Cohn, 1976). Membrane area is undoubtedly regulated by a balance between insertion and retrieval processes, either of which could be altered following adherence. It should also be noted that small and large cells do not necessarily represent two different subpopulations of cells. The J774 cell line is continuously dividing, and morphology may vary with cell cycle. For example, it is generally known that cells 'round up' during mitosis. The variability of membrane properties with cell cycle was not addressed here, although it is an interesting question that bears further study.

Membrane potential also increased with time after adherence, from −42 to −58 mV. Our results are consistent with those of Sung et al. (1985) who used ^{3}H-tetraphenylphosphonium to measure the membrane potential of J774 cells and found that it increased from about −35 to −70 mV between 1 and 8 hr after plating. It is unclear whether there is a significant increase in membrane potential between 0 and 1 hr after plating. Membrane potential values of suspended J774 cells, acquired using indirect probes, have been reported to be −15 mV (Sung et al., 1985), −25 mV (J774.2 cells; Ehrenberg et al., 1988), and −36 mV (J774.2 cells; McCaig & Berlin, 1983), and represent the average V_m of a large population of cells. The latter value is not very different from the average V_m determined in this paper for adherent cells (−42 mV) shortly after plating. Given the variability in the V_m values reported for suspended cells, and the unavoidable delays in measuring V_m after adherence, this study could not resolve whether or not there is a significant hyperpolarization of J774 cells immediately after plating. However, it does demonstrate that membrane potential was positively correlated with G_K, that is, cells with larger G_K were more negative. G_K increased significantly with time after adherence, while G_L did not. We have no evidence that any other conductance was modulated following adherence. Thus, it is likely that the increase in G_K accounts for the observed increase in V_m. We cannot exclude the possibility that part of the hyperpolarization following adherence is due to an increase in the activity of an electrogenic Na^+/K^+ pump, which is known to contribute −7 and −11 mV to the resting membrane potential of mouse spleen macrophages (Gallin & Livengood, 1983) and human monocytes (Ince et al., 1987), respectively. Increased pump activity might also lead to changes in $[K]_i$ and thus E_K. Our value of E_K is derived from measurements of $[K]_i$ in suspended J774 cells (Melmed, Karanian & Berlin, 1981; Sung et al., 1985), and is approximately −90 mV. However, even if resting V_m was determined solely by E_K, $[K]_i$ would have to double to shift E_K sufficiently to account for the observed increase in V_m.

It is important to note that, although G_K and V_m are positively correlated in J774 cells under a wide variety of conditions, the fact that resting V_m is considerably more positive than our assumed valued for E_K indicates the presence of a depolarizing conductance, such as sodium (E_{Na} = +70 mV, Sung et al., 1985) or chloride (E_{Cl} = −23 mV, Melmed et al., 1981). In our previous studies of J774 cells (Gallin & Sheehy, 1985; Gallin & McKinney, 1988), where more negative resting V_m values were reported (−70 to −80 mV), the contribution of other conductances to resting V_m was probably underestimated. This was because we tended to record from larger, more spread cells, and to assume that more positive resting V_m values were due to cell damage. Although we do not know what the ionic composition of the leak conductance is at this time, further dissection of this current may explain why the resting V_m is more positive than E_K.

Protein synthesis inhibitors have been shown to block the adherence-induced expression of the surface antigen FMC17 (recently classified as CD14; Triglia et al., 1985) and the transient increase in the surface expression of Ia-antigen in human monocytes that occurs during the first 12 hr in culture (Smith & Ault, 1981). In our studies, the protein synthesis inhibitor cycloheximide did not block the initial (0 to 2 hr) expression of G_K in adherent cells, but did inhibit the subsequent increase in channel density that occurred 2 to 8 hr after adherence. Increases in V_m and C_m were also inhibited. Because we did not record from cells which had been plated in cycloheximide for >8 hr, it was not possible to evaluate whether or not the increase in G_K which had occurred by one day after adherence requires further protein synthesis. In addition, because the time course of expression of G_K in J774.1 cells was similar to the time course of expression of the FMC17 antigen in human monocytes, we tested the effect of an antibody to the FMC17 antigen on G_K. However, no effects on whole-cell or single-channel K_i currents were observed.

Since 'primed' or 'activated' macrophages often exhibit increased spreading on surfaces (Pabst & Johnson, 1980) and since the degree of spreading in adherent J774.1 cells varied tremendously, it was also relevant to determine whether the membrane properties of very spread cells were different from those of nonspread cells. Spread cells had more negative resting membrane potentials than round

cells, both initially and one day after plating. Differences were also noted for the two groups of cells in their patterns of change after adherence. Only rounded cells increased G_K after adherence, and only spread cells increased capacitance. Thus, very spread cells inserted additional membrane area with time after adherence, but the increase in membrane area was not associated with a large increase in K_i channel density. The fact that G_K and capacitance varied independently of one another indicates that the increase in membrane area and expression of inwardly rectifying K channels were not necessarily linked. Final K_i channel density is determined by a balance between channel insertion or activation and channel internalization or inactivation. Our data do not differentiate between any of these processes.

The finding that spread cells did not show an increased density of K_i channels after adherence was consistent with the observation that exposing cells to LPS (which also increases cell spreading) did not increase the density of K_i channels. In fact, LPS-treated cells had significantly lower G_K values than control cells plated for comparable times. This is due in part to the increased capacitance of LPS-treated cells compared to controls, a result of the fact that LPS-treated cells are still growing, even though cell division has ceased.

Although the density of K_i channels in LPS-treated cells was lower than in control cells, resting V_m values were comparable. Clearly, there must be other differences between the two groups of cells to account for this result. Possible explanations are that LPS-treated cells have (*i*) a higher K permeability ratio (*ii*) a more negative E_K and/or (*iii*) increased electrogenic pump activity. However, like control cells, there was still a clear correlation between G_K and V_m in LPS-treated cells. Also, LPS-treated cells exhibited the same *increase* in K_i channel density, resting V_m, and capacitance following adherence as control cells did. Finally, in human monocytes, LPS has been reported to increase the percentage of cells expressing the transient outward K current (Jow & Nelson, 1989). In our studies, the K_v current was present in about 5% of the cells, but its expression was not increased by LPS.

In summary, the membrane properties of J774.1 cells change significantly after adherence, after exposure to LPS, and with cell morphology. Adherence is specifically correlated with increased K_i channel density, but only for rounded and not for spread cells. Spread cells rapidly increased their membrane area after adherence, but did not concomitantly increase K_i channel density. J774.1 cells treated for 24 hr with LPS were similar to spread cells in that they had increased capacitance but not increased K_i channel density compared to controls. G_K was positively correlated with V_m in both control and LPS-treated cells. Thus, the increase in G_K after adherence can account for the hyperpolarization which J774.1 cells undergo after plating, and an increase in surface membrane area is not necessarily linked to an increase in K_i channel density.

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