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Development of a delayed outward-rectifying K^+ conductance in cultured mouse peritoneal macrophages

(patch clamp/single K^+ channels/phagocytic cells)

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ABSTRACT **Patch clamp techniques were used to study ionic currents in cultured mouse peritoneal macrophages. Whole-cell voltage clamp studies of cells 1–5 hr after isolation showed only a high-resistance linear membrane. After 1 day in culture, 82 of 85 cells studied had developed a voltage- and time-dependent potassium (K^+) conductance similar to the delayed outward rectifier in nerve and muscle cells. The current activated when the membrane was depolarized above −50 mV. The sigmoidally rising current rose to a peak at a rate that increased with depolarization. Inactivation proceeded exponentially with a time constant of ≈450 ms. Recovery from inactivation was slow (τ = 12 s). The reversal potentials for varying extracellular K^+ concentrations followed the Nernst predictions for a K^+-specific channel. The conductance was blocked by extracellular 4-aminopyridine and by intracellular tetraethylammonium chloride, barium, and cesium. Single-channel K^+ currents comprising this net current had a conductance of 16 pS, exhibited bursting behavior, and inactivated with time. No inward currents were ever detected in macrophages cultivated for up to 4 days. Short-term exposure to chemoattractant and transmitter agents failed to activate an inward current. Macrophages may change their membrane electrophysiological properties depending on their state of functional activation. We postulate that the K^+ conductance develops prior to depolarizing conductances involved in the macrophage's immunological functions.**

Ionic membrane currents in macrophages may serve as signals between membrane–ligand interactions and cellular reactions such as phagocytosis of microorganisms. Cultured macrophages derived from human monocytes have an excitable membrane (1) and changes in membrane potential are among the earliest detectable events upon stimulation of phagocytosis (2). So far, direct membrane potential or current measurements have been made with the use of intracellular glass microelectrodes (1, 3, 4). This type of electrode, however, seriously hampers the study of ionic currents in small cells such as macrophages (3). Therefore, we have applied the patch clamp technique (5), which allows high resolution of ionic currents in small cells (diameter, <20 μm) up to high frequencies (<10 kHz). We report here that on the first day after isolation, mouse peritoneal macrophages develop an outward-rectifying potassium (K^+) conductance, similar to the delayed rectifier in nerve (6–8) and skeletal muscle membranes (9). The single channels underlying this conductance exhibit a linear current-to-voltage relationship and have a conductance of about 16 pS. Since Na^+ or Ca^{2+} currents could not be detected in these cells up to 4 days after isolation, we suggest that this conductance is the first conductance expressed in the development of macrophage excitability.

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MATERIALS AND METHODS

Resident macrophages were isolated from the peritoneal cavity of male NMRI mice of 20–22 g (Charles River Wiga GmbH, Sulzfeld, Federal Republic of Germany) as described elsewhere (10). Immediately after isolation the cells were spheroid, with diameters of 10–20 μm. The macrophages were cultured in plastic Petri dishes (Falcon) for up to 4 days at 37°C in a bicarbonate-buffered medium (pH 7.2; medium 199; GIBCO), supplemented with 20% fetal calf serum (GIBCO). Most experiments were performed at room temperature (22°C), but, for comparison, a few were made at 34–37°C. Before the experiments, the Petri dishes were washed and a standard extracellular salt solution was added (composition in mM: NaCl, 140; KCl, 2.8; $MgCl_2$, 2.0; $CaCl_2$, 1.0; and Hepes/NaOH, 10.0, pH 7.2.). The cells adhered well to the bottom of the dish. Giemsa-stained preparations fixed immediately after an experiment with methanol showed >99% macrophages. The standard intracellular salt solution, used to fill pipettes prior to whole-cell recordings (11), contained 140 mM KCl, 2.0 mM $MgCl_2$, 1.0 or 0 mM $CaCl_2$, 11.0 or 1.0 mM EGTA, and 10.0 mM Hepes/KOH (pH 7.2). In a few recordings KCl was replaced with KF. All drugs were obtained from Sigma. Experiments on one culture dish never lasted longer than 2 hr.

RESULTS

Whole-Cell Recordings. In most cultures "giga-seals" (5) were easily obtained and maintained for up to 30 min at room temperature. After 1–4 days of culture, whole-cell membrane current responses upon voltage clamp steps revealed the existence of a delayed outward-rectifying K^+ conductance in 82 of 85 cells tested (Fig. 1 *a* and *c–e*). This conductance developed on the first day after isolation, since 1–5 hr after isolation, 15 of 18 cells exhibited only a linear leakage resistance of >10 GΩ (Fig. 1*b*). In cells cultured >24 hr, resting potentials measured immediately after establishing whole-cell recording conditions were in the range of −80 to −90 mV. Surprisingly, in none of these cells were other voltage- and time-dependent conductances found in the voltage range of −200 to +40 mV.§

The K^+ conductance activates in the voltage range above −50 mV (Fig. 1 *a*, *c*, and *e*). Both activation and deactivation kinetics are voltage dependent. Upon depolarizing steps from a holding potential of V_h = −80 to −40 mV the current reaches its maximum in about 200 ms (Fig. 1*a*), whereas steps to +40 mV cause complete activation within 6 ms (Fig. 1*a*). Activation is usually followed by a single exponential

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§In some cells a slowly developing outward K^+ current was observed at voltages in the range above +40 mV. This resembled the Ca^{2+} and voltage-activated K^+ current (12), but we did not study this current in detail.

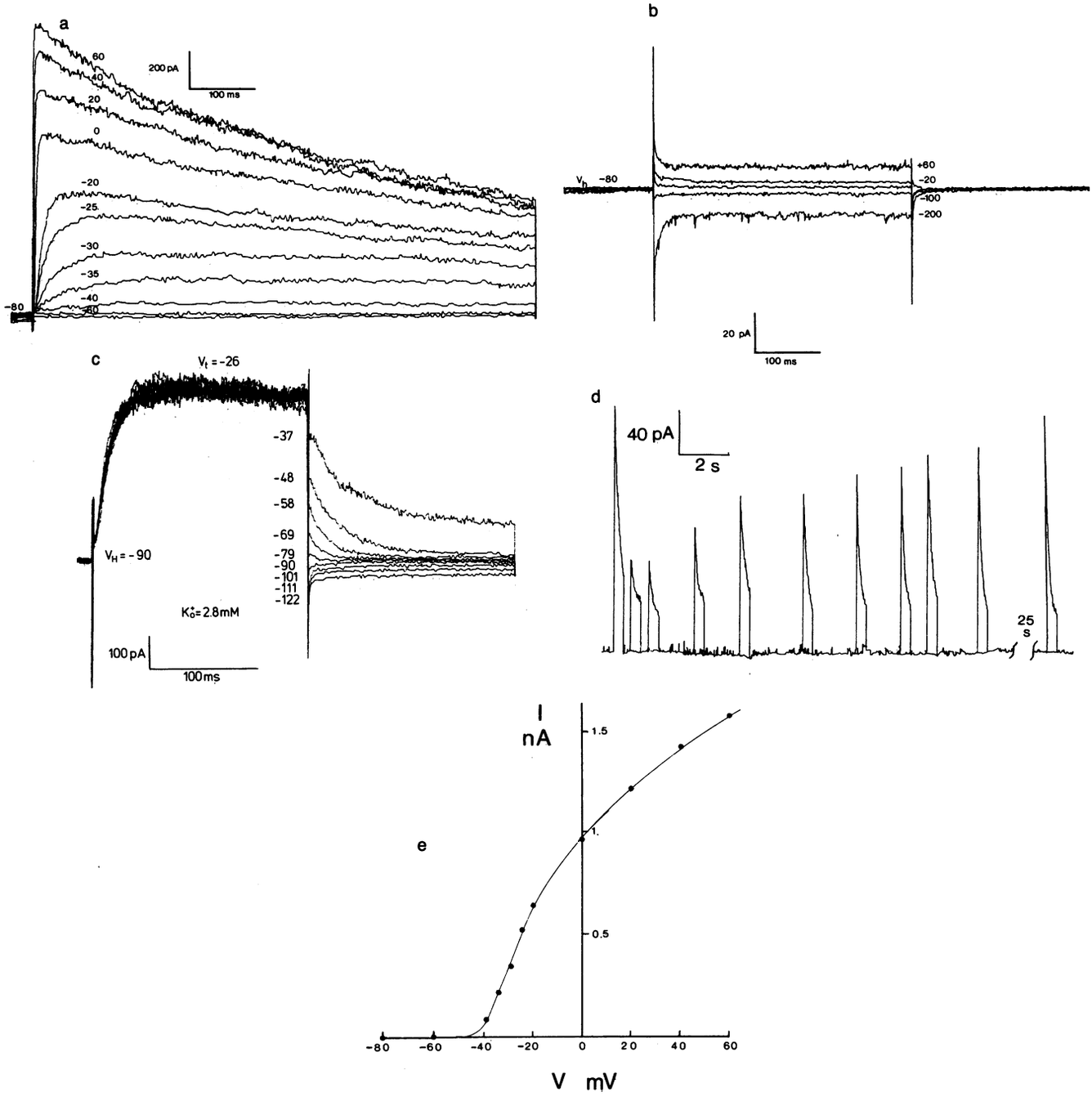

FIG. 1. Time- and voltage-dependent properties of the delayed outward-rectifying K^+ conductance of mouse peritoneal macrophages. Whole-cell current recordings under voltage clamp were at holding potential $V_h = -80$ mV. (*a*) Time course of activation of outward-going currents (cell of a 3-day culture) upon voltage steps to the depolarized values indicated (mV). To allow recovery from inactivation from previous pulses, 40-sec intervals separated test pulses. Membrane capacitance of this cell was 9.4 pF; series resistance was 4 MΩ. (*b*) Absence of voltage- and time-dependent currents in a cell 3 hr after isolation. Capacitive transients were not subtracted. Membrane capacitance was 7.5 pF; membrane resistance was 14 GΩ. (*c*) Deactivation of the outward current at different potentials (cell from 1-day culture), measured in a two-pulse voltage clamp protocol. The current was activated by 400-ms (duration) voltage steps to −26 mV at regular intervals of 6 s. Deactivation was initiated by steps back to different more negative potentials. Extracellular K^+ concentration was 2.8 mM. The tails reversed at approximately −90 mV, close to the calculated $E_K = -98.5$ mV. Deactivation became faster at more negative potentials. Similar experiments in 14 mM and 28 mM K^+ gave reversal potentials of −55 and −37 mV, respectively. (*d*) Slow recovery from inactivation (cell from 2-day culture). Voltage pulses were from −90 to −10 mV and had a duration of 400 ms. Test pulses were applied with variable intervals after a conditioning pulse, which followed the previous test pulse by 40 s (to allow complete recovery). Superimposed sweeps are given from oscilloscope tracings triggered by the conditioning pulses. (*e*) Peak current (●) (corrected for leakage) as a function of membrane potential obtained from the experiment illustrated in *a*. Membrane conductance (mainly leakage) at −80 mV was 3×10^{-10} S. Membrane conductance (mainly K^+ conductance) at complete K^+ activation (above 0 mV) was about 10^{-8} S. Assuming a specific membrane capacitance of 1 $\mu F/cm^2$, these values imply for this cell (membrane capacity = 9.4 pF) that the specific membrane resistance was about 30 $k\Omega/cm^2$ at −80 mV and 1 $k\Omega/cm^2$ at +60 mV.

inactivation process with a time constant $\tau_i = 463 \pm 213$ ms (mean ± SD; eight cells), virtually independent of voltage in the range of −30 to +40 mV. For example, single exponential fits to the tails of the records in Fig. 1*a* over an extended period of time (1 s) resulted in $700 < \tau_i < 760$ ms for this voltage range. This τ_i did not change during the experiment,

despite the variability in τ_i between cells. As Fig. 1*a* illustrates, the onset of inactivation is masked by the slow onset of activation at voltages more negative than −30 mV. Other experiments in which depolarizing steps were applied from various holding potentials less negative than V_h = −80 mV showed that significant inactivation does not occur below −60 mV and that steady-state inactivation is >90% above −10 mV. Reversal potentials (18 observations from seven cells; see Fig. 1*c*) follow a Nernst slope of 55.4 mV per decade as extracellular K^+ concentration was varied from 2.8 to 28 mM. Thus, this conductance is selective for K^+ ions.

Fig. 1*c* also illustrates that deactivation is voltage dependent. At −100 mV deactivation occurs within 40 ms, whereas at −50 mV deactivation is complete within 100 ms. Recovery from inactivation is complete in ≈40 s (τ = 12 s, Fig. 1*d*). A plot of the peak currents against the test potentials (Fig. 1*e*) exhibits the characteristic outward-rectifying nature of the macrophage membrane. Maximal K^+ conductance $\bar{G}_K$ (obtained after subtracting leakage) occurs above 0 mV and is, in general, $<10^{-8}$ S (see Fig. 1*e*). Obviously, the *I–V* curve would not exactly give steady-state K^+ conductance activation since activation is partly masked by inactivation at low depolarizing voltage steps. Analysis of the current records on a fast time scale, while making certain to separate capacity transient and current onset, showed that the current onset was sigmoidal (see Figs. 1*c* and 2*b*). Thus, a Hodgkin–Huxley type of description (6) of the K^+ conductance by $G_K = \bar{G}_K [n(t)]^x$ requires $x \geq 2$.

The conductance we describe as the delayed rectifier is blocked by tetraethylammonium chloride, 4-aminopyridine, and intracellular Ba and Cs. Tetraethylammonium blocks the channel preferentially from the intracellular side of the membrane (Fig. 2*a*). The current is blocked by 5 mM extracellular 4-aminopyridine (Fig. 2*b*), 5 mM intracellular Ba, or CsCl replacement for KCl. Block of the delayed rectifier by these substances has been described elsewhere (13). Under these conditions no other voltage- and time-dependent currents (for example, Na^+ or Ca^{2+}) were found in the voltage range of −150 to +40 mV (see Fig. 2*c*) nor did we find other conductances or significant changes of the voltage-dependent K^+ conductance after addition of 2 mM *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine (a synthetic chemoattractant), 20 mM histamine, 20 μM bradykinin, or 50 μM acetylcholine. At 34–37°C qualitative behavior of the outward-rectifying K^+ conductance was the same, but activation was at least two times faster.

Single-Channel Recordings. Current records from outside-out patches (5) showed the quantal nature of the microscopic currents underlying the macroscopic current (Fig. 3*a*). For an outside-out patch containing several channels, depolarizing voltage steps applied from V_h = −80 mV to values at which the outward-rectifying K^+ current activates caused qualitatively similar time-dependent currents (*Inset* in Fig. 3*b*) as in whole-cell records. The *I–V* curve (Fig. 3*b*) of the single-step currents is linear in the voltage range of −40 to +70 mV. An *I–V* curve collected from seven experiments has a slope of 15.7 ± 5.0 pS at asymmetric K^+ concentrations (140 mM in the pipette; 2.8 mM extracellularly) and extrapolates to a reversal potential of −89.3 ± 15.3 mV (mean ± SD; 236 data points), not far from the expected E_K = −98.5 mV. Thus, these channels are K^+-specific channels. Inactivation proceeds, at least in part, through a decline in probability of channel opening, as shown for Na^+ channels in muscle cells (14). No change in channel amplitude was seen in patches with only one channel. The similarity between time- and voltage-dependent behavior of the K^+ channels and the K^+ conductance and the absence of other time- and voltage-dependent channels and conductances in the voltage range of −100 to +40 mV imply that the observed K^+ channels constitute the observed K^+ conductance. Although we have not described channel kinetics here, there are at least two closed states, since channel openings occur in bursts with rapid transitions to short-lived closed states during bursts (Fig. 3*a*).

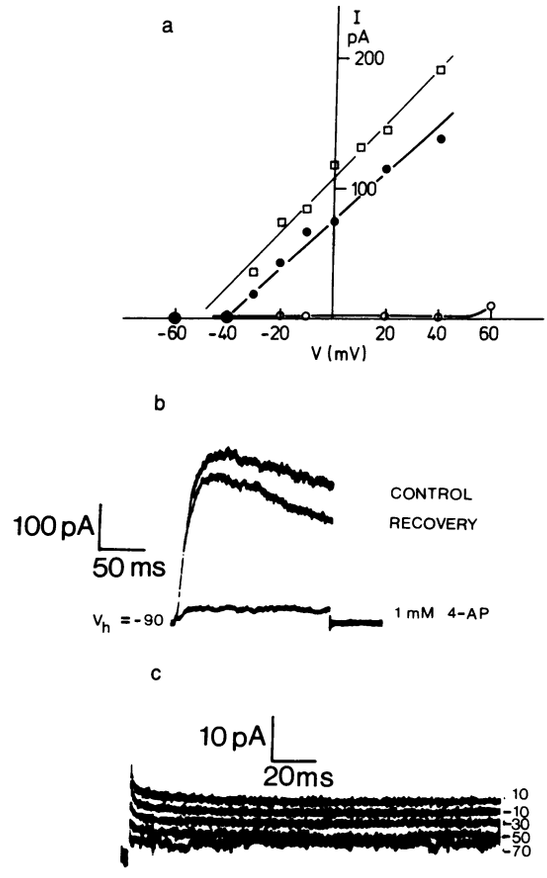

FIG. 2. Effects of K^+ conductance blockers. Whole-cell recordings under voltage clamp were at holding potential V_h = −80 mV (*a*) and V_h = −90 mV (*b* and *c*). (*a*) Partial block of K^+ current (□, control) by 10 mM extracellular tetraethylammonium (●) in a cell of a 2-day culture. Membrane capacitance was 5.7 pF. In another cell, where 10 mM tetraethylammonium was in the intracellular pipette solution, the K^+ current was completely blocked (○). (*b*) Effect of 1 mM 4-aminopyridine (4-AP) on the current response to 200-ms (duration) voltage steps to −30 mV (cell from 1-day culture). The depolarizations were applied at 6-s intervals. The record labeled 1 mM 4-AP was made 5 min after addition of the drug by perfusion. The recovery record was made after a 10-min wash. The initial effect of 4-aminopyridine was an increase in the speed of inactivation (not shown). The instantaneous effect of the voltage step was the same in the control and in the 4-aminopyridine record. (*c*) Absence of inward currents in a cell with a 120 mM Cs/20 mM tetraethylammonium intracellular solution (cell from 1-day culture). Voltage steps to the indicated potentials were applied every 5 s (step duration, 200 ms). No K^+ conductance or any other voltage- and time-dependent conductance was visible in these high-gain records, covering the normal voltage range of activation of the outward-rectifying conductance. Leakage conductance (2×10^{-10} S) was linear between −130 and +10 mV.

DISCUSSION

We report a voltage- and time-dependent K^+ conductance in macrophages similar to that present in nerve and muscle cells. This delayed outward-rectifying conductance develops on the first day after isolation without development of inward currents. Activation and deactivation kinetics are voltage dependent, but inactivation, which is a much slower process, is voltage independent.

Ca^{2+} currents show a tendency to decline with time, if

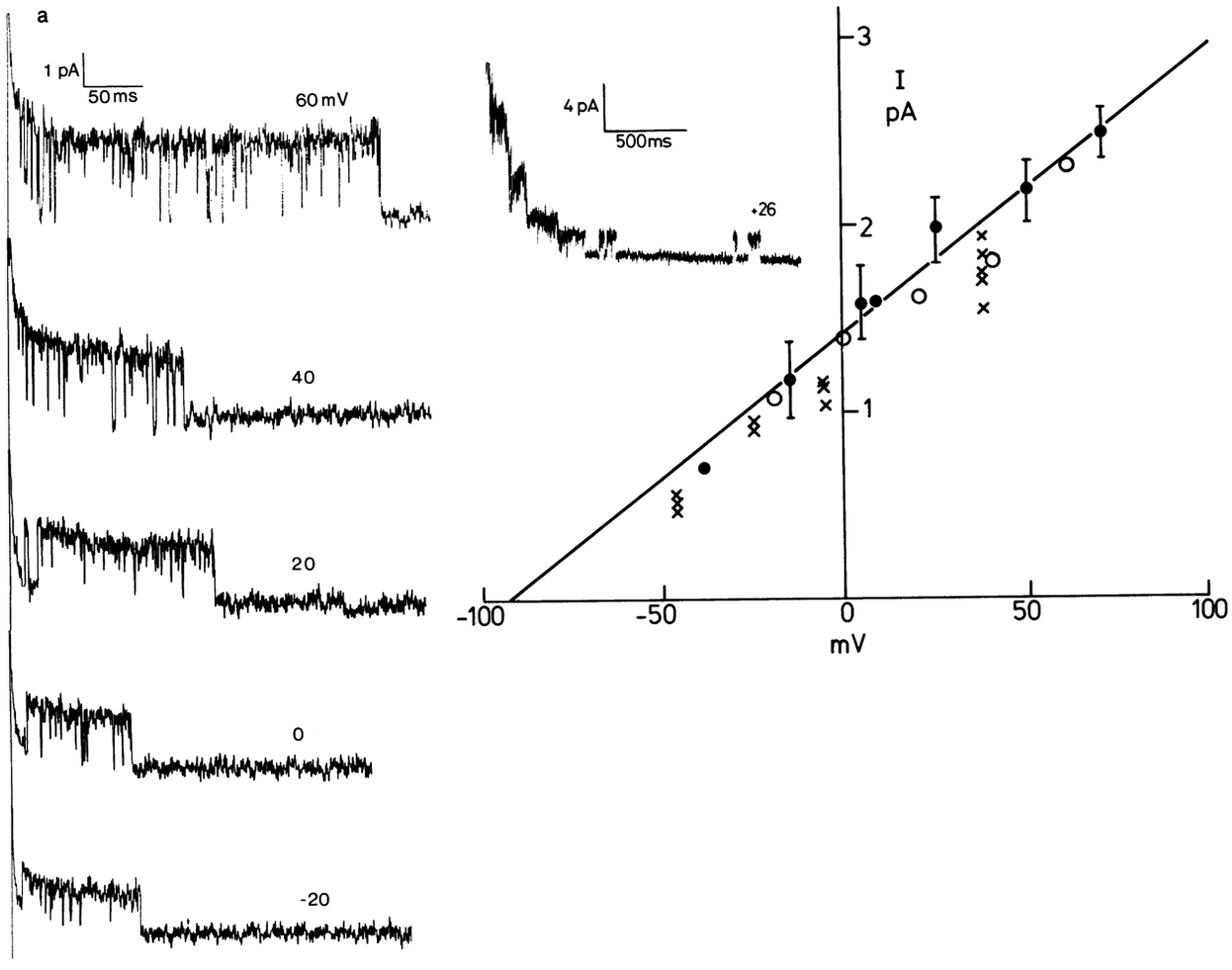

FIG. 3. (*a*) Current responses of an outside-out patch to the depolarizing voltage steps indicated from $V_h = -80$ mV. $K_i = 140$ mM; $K_o = 2.8$ mM. Single channel amplitudes are plotted in *b* (○). (*b*) Single-channel *I–V* plot from three outside-out patches. The least-squares-fit curve applies to a patch with several channels. The data points (●) are means, with bars giving the range of variation (31 observations; 3-day culture). The curve extrapolates to a reversal potential $E_r = -90.8$ mV and has a slope $\gamma = 16$ pS. Data points (×, ○) are from patches with only one channel and resulted in a $E_r = -93$ (×) or -97 (○) mV and a $\gamma = 13$ (×) or 14 (○) pS, respectively (fitted curves not shown). (*Inset*) A patch with at least 10 channels that open and then inactivate with time after the voltage step to +26 mV.

measured in internally perfused cells (15). In our cells, it is unlikely that such a "rundown" eliminated Ca^{2+} currents within the few seconds' time needed to establish whole-cell recording and depolarize to potentials at which Ca^{2+} currents would be observed. In chromaffin cells, the fastest Ca^{2+} current rundown occurred over several minutes (15). However, we cannot exclude the possibility that rundown of Ca^{2+} channels in these cells may be much faster than in other cells.

This study shows the high-resistance nature of the macrophage membrane as predicted from analysis of microelectrode impalements (3). Membrane resistance varies between 10^8 and 10^{10} Ω, depending on the presence of the K^+ conductance and its degree of activation (and inactivation). This range includes values much higher than ever found with microelectrodes (1, 3, 4). We have also shown that the patch clamp technique allows the study of macrophages at very early stages of *in vitro* culture as opposed to macrophages treated in various ways or cultured for weeks to increase cell size for microelectrode studies (3, 4).

Our results pose questions as to the role of this K^+ current in macrophage function, since resident mouse peritoneal macrophages under our conditions of measurement do not have inward voltage- and time-dependent conductances (e.g., for Na^+ or Ca^{2+}) within 4 days after isolation. However, human macrophages in culture can produce action potentials (1) and thioglycolate-elicited mouse peritoneal macrophages (long-term cultured) may exhibit an inward-rectifying K^+ conductance (4). Therefore, macrophages may be able to change their membrane electrophysiological properties, depending on their state of functional activation. We hypothesize that the K^+ conductance described in the present study develops prior to future depolarizing events or incorporation of inward channels and that this conductance is involved in immunological functions of macrophages such as phagocytosis.

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