J. Membrane Biol. 97, 251–258 (1987)

The Journal of
Membrane Biology

Ionic Channels and Membrane Hyperpolarization in Human Macrophages

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Summary. Microelectrode impalement of human macrophages evokes a transient hyperpolarizing response (HR) of the membrane potential. This HR was found to be dependent on the extracellular concentration of K^+ but not on that of Na^+ or Cl^-. It was not influenced by low temperature (12°C) or by 0.2 mM ouabain, but was blocked by 0.2 mM quinine or 0.2 mM Mg^{2+}-EGTA. These findings indicate that the HR in human macrophages is caused by the activation of a K^+ (Ca^{2+}) conductance. Two types of ionic channels were identified in intact cells by use of the patch-clamp technique in the cell-attached-patch configuration, low and high-conductance voltage-dependent K^+ channels. The low-conductance channels had a mean conductance of 38 pS with Na^+-saline and 32 pS with K^+-saline in the pipette. The high-conductance channels had a conductance of 101 and 114 pS with Na^+- and K^+-saline in the pipette, respectively. Cell-attached patch measurements made during evocation of an HR by microelectrode penetration showed enhanced channel activity associated with the development of the HR. These channels were also high-conductance channels (171 pS with Na^+- and 165 pS K^+-saline in the pipette) and were voltage dependent. They were, however, active at less positive potentials than the high-conductance K^+ channels seen prior to the microelectrode-evoked HR. It is concluded that the high-conductance voltage-dependent ionic channels active during the HR in human macrophages contribute to the development of the HR.

Key Words ionic channels · patch-clamp technique · human macrophages · calcium-dependent potassium conductance · membrane potential

Introduction

Mononuclear phagocytes originate in the bone marrow, enter the blood circulation as monocytes and migrate to the tissues where they become macrophages. These cells play an important role in host defence because of their ability to recognize, phagocytose, and kill invading pathogenic microorganisms intracellularly. There is increasing evidence suggesting that membrane electrophysiological processes play an important role in the function of mononuclear phagocytes [2, 3, 9, 14, 22, 28, 38, 39]. These studies have demonstrated membrane hyperpolarizations in macrophages performing such functions as phagocytosis [22], chemotaxis [9] and Fc-receptor ligation [38]. As shown by earlier studies oscillatory membrane hyperpolarizations can be induced by microelectrode penetration of murine macrophages and fibroblasts and are caused by the activation of a calcium dependent potassium conductance [15].

Application of the patch-clamp technique to mononuclear phagocytes has shown that these cells possess ionic channels [7, 19, 28, 35, 39, 40]. Recently we showed by use of cell-attached patch measurements that phagocytosis by human macrophages is accompanied by an increase in ionic channel activity [14]. To gain more insight into the ionic processes underlying phagocytosis, therefore, more knowledge is needed about the membrane electrophysiological properties of human macrophages.

The present study was performed to establish the nature of ionic channels present in intact human monocyte-derived macrophages and the role of these channels in the hyperpolarizing response (HR). Microelectrode impalements were used to evoke the hyperpolarizing response and to determine the ionic properties of the HR. The cell-attached patch configuration was chosen because it would make it possible to identify the channels present in the intact cell membrane. The peak of the impalement transient measured in the first few milliseconds of microelectrode penetration was used to account for the resting membrane potential in these measurements [17]. Finally, microelectrode penetrations were performed during cell-attached patch recordings to find out whether the hyperpolarizing response results from channel activity. Preliminary

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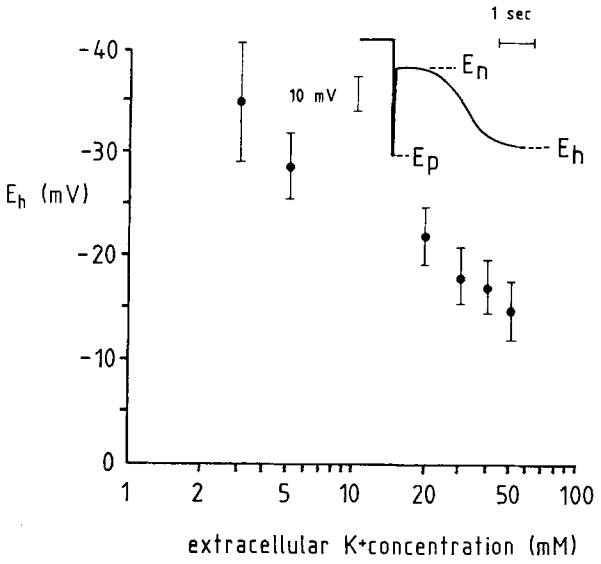

Fig. 1. Dependence of the hyperpolarizing response (HR) of human monocyte-derived macrophages on the extracellular potassium concentration. The HR is expressed as maximum hyperpolarization (E_h). Each point represents the mean E_h value ± SD of 30 records. The inset shows a schematic diagram of a typical potential transient as seen upon impalement of a human macrophage. The rapid potential transient upon microelectrode entry, with its maximum level E_p, is visible as a negative spike. Then follows, from E_n, the hyperpolarizing response reaching a maximum value E_h

results of parts of this work have appeared elsewhere [19].

Materials and Methods

Mononuclear Phagocytes

Human blood monocytes were isolated from the blood of healthy donors on Ficoll-Hypaque as described elsewhere [25]. Monocytes were cultured for 1 to 3 weeks on glass coverslips as already described [20]. During one to three weeks of culture the monocytes increase in size and become macrophage-like cells. Such monocyte-derived macrophages were used in the present study.

Electrophysiology

Electrophysiological experiments were done on glass-adherent cells, with the use of a Teflon culture dish which, when transferred to an inverted microscope, permitted use of 100× oil-immersion optics [18]. During the experiments the cells were bathed in a Na^+-saline solution composed of (in mM) 150, NaCl; 3, KCl; 1, $MgCl_2$; 4, $CaCl_2$; and 10, HEPES-NaOH (pH 7.2). Microelectrode measurements were made as described previously [17] and performed at room temperature. Capacitance compensation was used to improve the response of the microelectrodes to time constants less than 0.1 msec. This is indispensable for measurement of the resting membrane potential of small cells by use of the peak value of the rapid potential transient seen in the initial milliseconds of microelectrode entry into the cell [17]. Patch pipettes were pulled from thin-walled borosilicate glass. The tips were flame polished (tip diameters 1 to 5 μm). Patch pipettes were filled with either the Na^+-saline solution or a K^+-saline composed of 143 mM KCl, 2 mM $MgCl_2$, 2 mM $CaCl_2$ 10 mM HEPES-KOH (pH 7.2) and 10 mM EGTA to buffer Ca^{2+} to intracellular levels. Gigaseals were made on cells according to Hamill et al. [12]. Current was measured with an *I-V* converter according to Hamill et al. [12]. Patch clamp measurements were recorded on tape (FM recorder) and visualized for analysis by an oscilloscope, a fast chart recorder or by use of a correlator (Type 3721A correlator, Hewlett-Packard, U.K.) from which amplitude histograms could be obtained. The percentage open time of channels in cell-attached patches was calculated from the area under the amplitude histograms using the equation

$$\frac{\sum_{n=1}^{N} (\text{time that } n \text{ channels are open})\ (n)}{(\text{total time})\ (N)} \times 100\%$$

and under the assumption that the total number of channels (N) in the patch could be determined by maximal stimulation (i.e., the voltage applied to the patch, above which no additional ionic channel is activated). Values are expressed as means ± SD.

Results

Membrane Potential Measurements

Microelectrode impalement of a human macrophage causes a rapid negative-going peak-shaped potential transient reaching a peak value (E_p) followed by a depolarization of the membrane to a level E_n (inset Fig. 1). This initial peak transient occurs as a result of the introduction of a transmembrane shunt resistance by the measuring electrode, as described elsewhere [15, 17, 20, 23]. The value of the peak potential (E_p) has been shown to provide a good estimate of the pre-impalement potential of the cell as shown by experiments where whole-cell current clamped human macrophages were impaled by microelectrodes [17]. After remaining momentarily at E_n, the membrane hyperpolarizes to a maximum potential E_h (inset Fig. 1). This hyperpolarizing response (HR) activated by the microelectrode is accompanied by a decrease in membrane resistance [15]. After reaching E_h, the membrane slowly depolarizes, accompanied by an increase of the membrane resistance [15], to a sustained steady-state potential (*not shown*, roughly equal to E_n). Since this steady-state potential mainly reflects microelectrode-induced shunt properties of the cells,

Table. Hyperpolarizing response of human macrophages under various experimental conditions

		E_h (mV)	SD	n
Na^+-saline		−35.1	12.0	61
Zero Na^+		−29.9	7.3	24
Low Cl^-	(10 mM)	−34.5	15.8	28
12°C		−27.8	17.0	23
Ouabain	(0.2 mM)	−28.8	12.9	38
EGTA-Mg^{2+}	(0.2 mM)	−14.3	4.9	30
Quinine	(0.2 mM)	−12.8	2.0	25

it was not taken into consideration [23]. Contrary to that found in mouse macrophages [15], human macrophages never exhibited oscillatory hyperpolarization upon microelectrode impalement. The mean values of the membrane potentials measured after microelectrode impalement in 61 cells were: E_p = −35.2 ± 11.2 mV, E_n = −15.1 ± 5.6 mV, and E_h = −35.1 ± 12.8 mV.

To establish the ionic properties underlying the HR in human macrophages, microelectrode measurements were performed in the presence of extracellular bathing solutions with different concentrations of K^+, Na^+, and Cl^- obtained by equimolar substitution of these ions by, respectively, Na^+, choline and SO_4^{2-} (with equiosmolar addition of mannitol in the latter case) in Na^+-saline. The results of these experiments showed a dependence of E_h on extracellular K^+ (Fig. 1) but not on Na^+ or Cl^- (Table). Blockade of the Na^+/K^+ pump by addition of 0.2 mM ouabain or measurement at low temperatures had no appreciable effect on the HR as judged from the value of E_h (Table). Extracellular Ca^{2+} was, however, required to elicit the HR, since no HR was observed upon addition of Mg^{2+}-EGTA (0.2 mM EGTA + 0.5 mM $MgCl_2$; Table). The HR was also blocked by 0.2 mM quinine (Table), a blocker of the $K^+(Ca^{2+})$ conductance in macrophages [32]. Taken together, these findings indicate that the $K^+(Ca^{2+})$ conductance is responsible for the microelectrode-induced HR in human macrophages.

Cell-Attached Patch Measurements

Cell-attached patch measurements under voltage clamp on human macrophages bathed in Na^+-saline were used to detect channel activity in the cell with its normal cytoplasmic contents. Open states of channels could often be distinguished from the closed states by the enhanced noise level seen in the open state. Multilevel current jumps reflected the presence of more than one channel in the patch. Equidistant peaks in amplitude histograms of such multilevel recordings indicated that several channels of the same conductance were present in the patch. Current-voltage relationships and the percentage open time were calculated from these amplitude histograms.

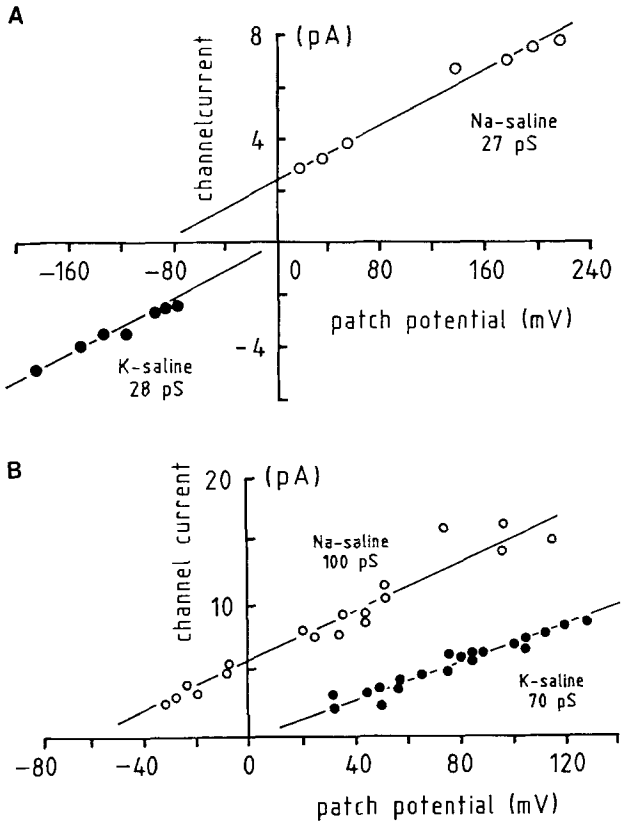

Fig. 2. Current-voltage relationships of two types of K^+ channels in cell-attached patches of human macrophages showing low conductance (*A*) and a high-conductance (*B*) channels. The patch potential was calculated from the pipette potential and the resting membrane potential (obtained from peak potential measurements). Channel conductances and pipette fillings are indicated

In cell-attached patch measurements the potential difference across the membrane patch (E_{pat}) is given by: $E_{pat} = E_m - E_{pip}$ in which E_m is the membrane potential and E_{pip} is the potential imposed by the voltage clamp on the pipette. To determine the relation between channel currents and the potential drop across the (cell-attached) membrane patch it is essential to know the resting membrane potential (E_m) [12]. Uncertainty about E_m, therefore, causes a serious limitation of the cell-attached patch tech-

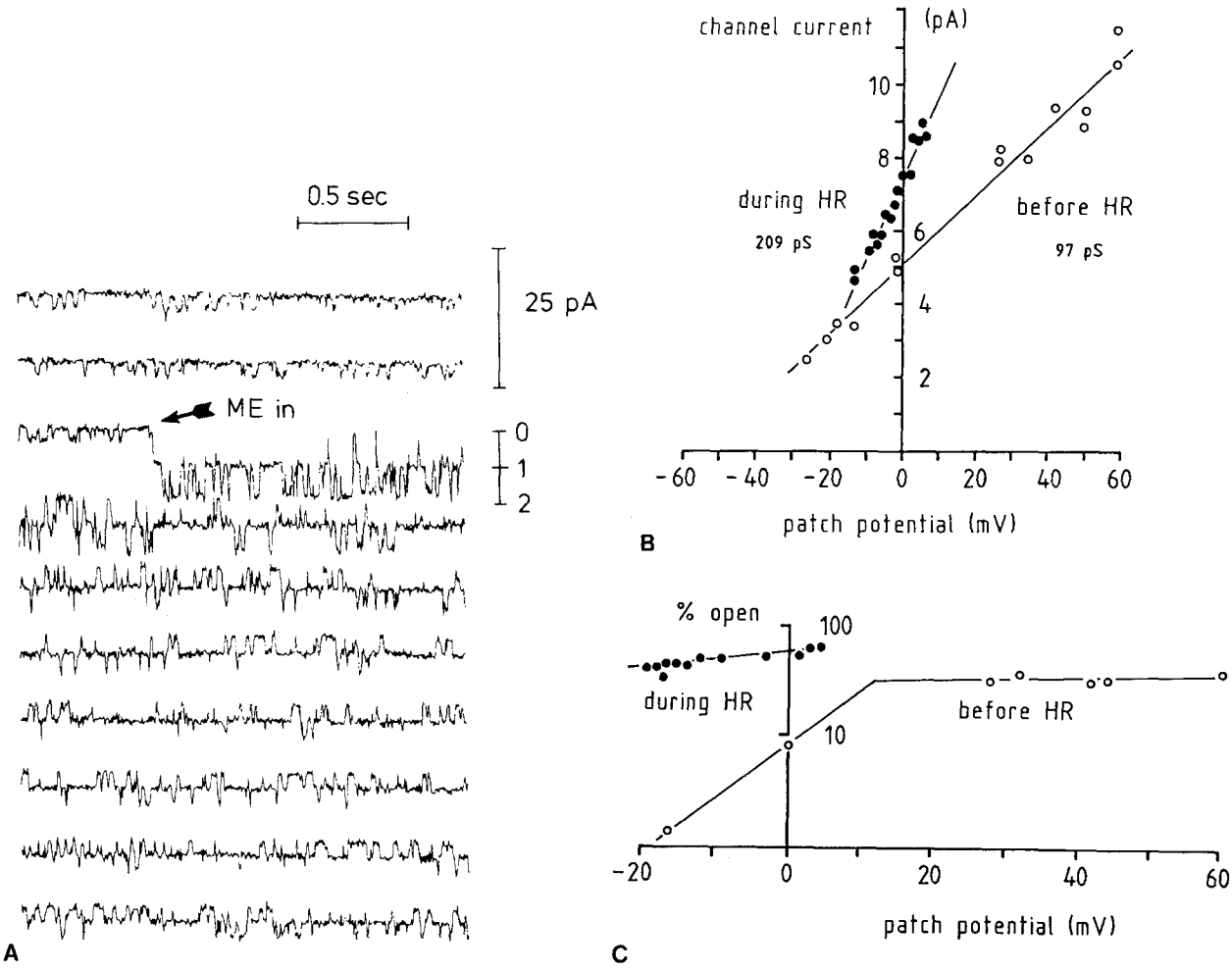

Fig. 3. Channel activity in a cell-attached patch upon microelectrode impalement of a human macrophage. (*A*) The HR evoked by the microelectrode is associated with a rise in channel activity. The patch pipette was filled with Na^+-saline and the pipette potential was held at −16 mV to depolarize the patch. The peak of the impalement transient measured in this cell by the microelectrode was −32 mV, and this value was used to calculate the patch potential before impalement. The 0-, 1-, and 2-channel current levels are indicated, with downward currents corresponding to outward currents from the patch. (*B*) The current-voltage relationship measured from channel openings prior to impalement showed a conductance of 97 pS, whereas that during the HR amounted to 209 pS. (*C*) The patch potentials at which the HR-associated channels are active (expressed in terms of the % time the channels are in the open state) are more negative than that of the high-conductance channels seen before the HR

nique. To solve this problem, we determined the peak potential measured by microelectrode impalement, as a measure of the E_m of the patched cells [17, 20].

Cell-attached patch measurements revealed two classes of ionic channels in human macrophages: low-conductance and high-conductance channels. With K^+-saline as pipette filling, the low-conductance channels appeared at negative patch potentials and had a conductance of 32 ± 10 pS (n = 9). The *I-V* relationship of these channels crossed the E_{pat} axis at a potential of −3 ± 24 mV (n = 9) (Fig. 2*A*). With Na^+-saline as pipette filling, low-conductance channels appeared at positive patch potentials and had a single channel conductance of 38 ± 10 pS (n = 6) and a reversal potential of −82 ± 10 mV (n = 6). Since this reversal potential is much closer to the reversal potential of K^+ than to that of Na^+ or Cl^- (based on intracellular ion concentration determinations, the reversal potential of K^+ is −96 mV [16] and of Na^+ and Cl^- is +47 and −11 mV, respectively [13]), it may be concluded that the small conductance channels predominantly conduct K^+ ions.

With K^+-saline as pipette filling, the high-conductance channel had a conductance of 114 ± 33 pS (n = 9) and activated with depolarization (Fig. 3*C*). The reversal potential of these channels amounted

to +5 ± 15 mV (n = 9). With Na^+-saline as pipette filling, the reversal potential of the high conductance channels amounted to −70 ± 10 mV (n = 6), which is not far from the equilibrium potential of K^+ (Fig. 2*B*), indicating that these channels are probably also K^+ channels. The channel conductance with Na^+-saline as pipette filling amounted to 100 ± 18 pS (n = 6). Since both channel types were present in the same patch in only 2 out of 48 cases, it may be concluded that the low-conductance channel is not a substate of the high-conductance channel.

Channels Associated with the Hyperpolarizing Response

To find out whether the hyperpolarizing response (HR) is associated with channel activity, cell-attached patch measurements were made during an HR evoked by microelectrode impalement. Impalement of patched cells led to enhanced channel activity accompanying the HR (Fig. 3*A*). Current-voltage relationships of channels during the HR (Fig. 3*B*) show that a high-conductance channel is involved in this response. With Na^+-saline as pipette filling, channels active during the HR had a reversal potential of −42 ± 11 mV, n = 5. This implies that this channel essentially contributes to the development of the HR, since the value of its reversal potential is more negative than E_h (Table). A similar reversal potential (−52 mV) was also found for the HR in earlier microelectrode studies on human macrophages [11]. Single-channel conductances measured during the HR with Na^+- or K^+-saline as pipette filling were similar with conductances of 171 ± 64 pS (n = 5) and 165 ± 60 pS (n = 4), respectively. In all cases where channel activity was measured during the microelectrode-evoked HR only the high-conductance K^+ channel was present under the patch prior to microelectrode penetration, suggesting that the presence of the high-conductance K^+ channel under the patch and the occurrence of channel activity during the HR seem to be related.

A plot of the percentage channel open time as a function of the membrane patch potential prior to and during the HR showed that the ionic channels associated with the HR were active at more negative potentials than that of the high-conductance K^+ channel active before microelectrode impalement (Fig. 3*C*). The channels active during the HR were voltage dependent as shown by the influence of the holding potential on the time course of channel activity with respect to the time course of the microelectrode-induced HR. At a holding potential of 0 mV, (i.e., patch potential equals membrane potential) the maximum channel activity either coincided with or slightly followed the HR (Fig. 4*A*; n = 4). Negative holding pipette potentials of −16 mV (n = 4) (which depolarized the patch) resulted in an earlier maximum channel activity, i.e., immediately after impalement and before maximum hyperpolarization (E_h) was attained (Fig. 4*B*). Furthermore, the observation that pulses applied to the patch pipette that depolarized the patch during the HR led to enhancement of channel activity, indicated that the channels during the HR are voltage dependent (*data not shown*).

Discussion

The main finding of the present study is that the hyperpolarizing response, present in many different cell types [4, 5, 11, 15, 21, 24, 26, 29, 33, 36, 37], is associated with the action of ionic channels. The conclusion that the microelectrode-induced HR in human macrophages is caused by the activation of a K^+ (Ca^{2+}) conductance is based on four observations: (i) the mean value of E_h in human monocytes is identical to that in other mononuclear phagocytes [20, 32]; (ii) the HR is accompanied by a decrease in impaled membrane resistance [5, 15, 33]; (iii) the amplitude of the HR is dependent on the extracellular K^+ concentration [1, 11, 33] and not on extracellular Na^+ or Cl^- [21, 30, 33]; and (iv) the HR is blocked by EGTA [11, 15] and quinine [1, 4, 6, 31, 32] but not by ouabain [21] or by temperatures around 12°C [29]. The observation that the $K^+(Ca^{2+})$ conductance is not active in mouse macrophages and fibroblast cell lines prior to microelectrode entry but is evoked by the penetration of the microelectrode [15] was confirmed for human macrophages by use of cell-attached patch measurements performed in the present study.

High and low conductance ionic channels were identified in intact human macrophages. The low-conductance channels found in this study have not been described before in human macrophages. The low-conductance channels measured in Na^+-saline are probably potassium channels and resemble the K^+ channels found in J774.1 macrophages [10]. The high-conductance K^+ channel measured in the cell-attached patch mode shows similarities with a class of $K^+(Ca^{2+})$ channels identified by use of cell-excised patch measurements in human monocyte-derived macrophages [8]. With Na^+-saline on the outside and K^+-saline on the inside of the patch (an excised patch configuration with the closest resemblance to an in situ measurement) this study found for the $K^+(Ca^{2+})$ channels a mean conductance of

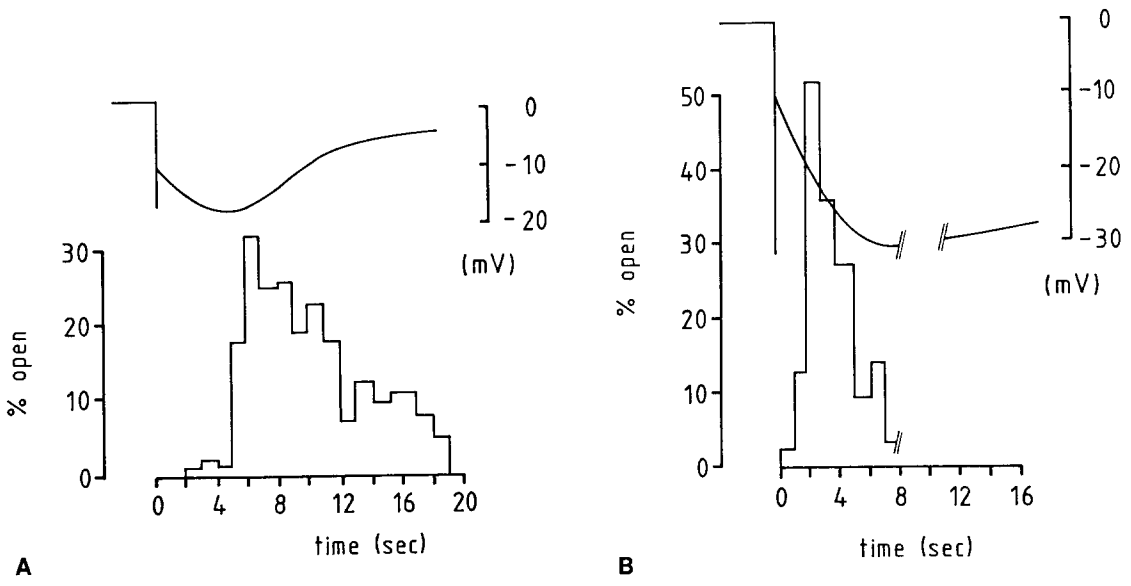

Fig. 4. The effect of the holding potential on the time course of channel activity (expressed as % open time) as measured by the patch pipette during the microelectrode-induced HR. The % open time of channels as function of time is shown below the HR as measured by the microelectrode. Na^+-saline was used as pipette filling. (*A*) With the patch held at the membrane potential (0 mV pipette potential), the average open time reaches a maximum somewhat after E_h has been reached. (*B*) Depolarizing the patch by 16 mV (−16 mV pipette potential) causes maximum channel activity to occur before E_h is reached

130 pS, which is close to the value of 100 pS found presently in the in situ measurements [8]. Furthermore, these $K^+(Ca^{2+})$ channels activated at positive patch potentials similar in value to those at which the high conductance K^+ channels were active.

The present study has established that ionic channel activity contributes to the HR. This conclusion is based on the finding that the reversal potential of the HR-associated channels is more negative than E_h. In L-cells (a murine fibroblast cell line with membrane electrophysiological properties similar to those of macrophages [32]) it has been suggested that the HR is caused by a K^+ carrier mechanism [34]. Calcium channel activity, expected from the role suggested for calcium channels in the HR [31] and from the report of action potentials in human macrophages [27], was not observed during or before the HR, probably because the measurement conditions were not favorable enough. Channel activity during the HR remains voltage sensitive, as indicated by the finding that its time course during the HR can be influenced by the holding potential (Fig. 4) and the depolarizing voltage pulses delivered through the patch pipette during the HR can enhance channel activity.

The finding that the pre-HR and HR-associated channels are both depolarization-activated channels and that the HR-associated channels always appeared in patches where high-conductance K^+ channels had been identified prior to the HR leads to the speculation that these channels might belong to the same class of channels. That the HR-associated channels are active at more negative potentials than the high-conductance K^+ ones seen prior to microelectrode penetration would be consistent with the findings that the HR is associated with a rise in intracellular Ca^{2+} concentration [15, 33] and that $K^+(Ca^{2+})$ channels activate at more negative potentials for higher concentrations of Ca^{2+} [8]. In this respect channel properties determined from cell-excised patch measurements on human macrophages [8] would be consistent with the cell-attached patch data in the present study.

Although there is some evidence indicating that the high-conductance K^+-conducting channels and the HR-associated channels might belong to the same class of channels, some evidence is also present suggesting that they are not. In general, for example, HR-associated channels had larger single-channel conductances than the high-conductance K^+ channels seen prior to microelectrode impalement. A shift in reversal potential of the channels upon microelectrode impalement also argues against the notion that the HR-associated channels and the other high-conductance channels are the same. However, the introduction of a microelectrode-induced transmembrane shunt upon impalement of the cell [15, 17, 23] could cause K^+ leakage from the cell, thereby shifting the reversal potential for K^+ to less negative values. More experiments

are needed, therefore, to establish the precise relation between the pre-HR channels and the channels associated with the HR.

The experiments described in this paper were carried out in the Department of Physiology, University of Leiden, The Netherlands.

The authors are grateful to Prof. R. Van Furth and Prof. A.A. Verveen for their continued support and critical reading of the manuscript. We also wish to thank Joke van de Gevel for culture of cells and Kees Versluis for electronic support.

C. Ince is supported by the Foundation for Medical Research (FUNGO), which is subsidized by The Netherlands Organization for the Advancement of Pure Research (ZWO).

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Received 5 January 1987; revised 9 April 1987