Activation of membrane outward currents by human low density lipoprotein in mouse peritoneal macrophages

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Summary. The aim of the present study was to search for electrophysiological effects of human lipoproteins on membrane currents in mouse peritoneal macrophages which had been cultured for 5 to 20 days. Whole-cell currents were recorded by using a voltage-clamp technique.

Low density lipoprotein (LDL, 100 µg/ml) increased a slowly activating nonspecific cation current ($i_{m}$) in the positive potential range to $244 \pm 23\%$ of the reference (test potential $+55$ mV, $n = 13$, $P < 0.005$). Augmentation of current resulted out of a negative shift of the activation curve along the voltage axis ($-22$ mV) and an increase of maximally available current.

Furthermore, LDL increased a rapidly activating outward current ($i_{o}$) at test potentials positive to the potassium equilibrium potential. At $+55$ mV $i_{o}$-amplitude increased to $165 \pm 14\%$ of reference ($n = 16$, $P < 0.005$). LDL-induced effects on $i_{o}$-current could be mimicked by application of the calcium ionophore A23187 ($1 \mu$mol/l) which led to an increase of $i_{o}$-current to $161 \pm 25\%$ of the reference (test potential $+55$ mV, $n = 11$, $P < 0.005$).

Acetylated-LDL (100 µg/ml, 5–15 min) produced no significant effect on the membrane currents under investigation.

Key words: Macrophage – Voltage-clamp – Ionic current – Low density lipoprotein – Acetylated low density lipoprotein

Introduction

Lipoprotein metabolism in macrophages has been extensively studied in recent years by biochemical methods (review: Brown and Goldstein 1983; Steinberg et al. 1989). Exposure of mouse peritoneal macrophages to human lipoproteins is one test system widely used in biochemical investigations. Mouse macrophages hardly take up native low density lipoproteins (LDL) and therefore are thought to express few if any LDL-receptors (Brown and Goldstein 1983). However, chemically modified LDL is taken up in large amounts by macrophages via endocytosis which is mediated by the so-called scavenger receptor(s) or acetyl-LDL receptor on the surface of mouse peritoneal macrophages (Sparrow et al. 1989; Steinberg et al. 1989). Intracellular cholesterol overflow is supposed to result in a conversion of macrophages to foam cells (Brown and Goldstein 1983; Goldstein et al. 1979).

In the present study we searched for electrophysiological effects of human acetyl-LDL or LDL on membrane currents in mouse peritoneal macrophages. A detailed review of ion channels existing in macrophages is given by Gallin (1991). In general no voltage-gated Na- or Ca-currents have been found in macrophages by direct electrophysiological measurements. Ion channels to be encountered in macrophages are: Fc-receptors, 3 different chloride passing channels, a non-selective cation passing channel and 5 different potassium channels. The potassium currents passing through the different channels show characteristics which are observed in excitable tissues as well: 1 inwardly rectifying K-current, 2 different transient outward currents and 2 Ca-activated outward currents (Gallin 1991).

We report here that lipoproteins do affect membrane currents in macrophages. LDL activates 2 different outward currents while acetyl-LDL had no effect on the currents under investigation.

Materials and methods

Resident macrophages were isolated from male NMRI-mice (40 g, Tiererversuchsanlage, Universität Düsseldorf, FRG) according to standard techniques. In short, animals were killed by cervical dislocation. DMEM-solution (10 ml, Dulbecco's mod. eagle medium) was injected into the abdominal cavity and after massage of the abdomen about 7 to 8 ml of cell-rich fluid was regained and centrifuged (1200 rpm, 10 min, Omnimug 2.0 RS, Heraeus, Hanau, FRG). The pellet was suspended in 10 ml DMEM, rewashed, and resuspended in culture medium.

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which was cumulated as follows (all ingredients from Gibco/BRL GmbH, Eggenstein, FRG): 500 ml DMEM plus 50 ml bovine fetal calf serum plus 5 ml sodium pyruvate (100 mmol/l stock) plus 5 ml L-glutamine (200 mmol/l stock) plus 5 ml MEM non-essential amino acids (100 x) plus 5 ml of a penicillin (5000 IU/ml)-streptomycin (5000 μg/ml) solution. Macrophages were plated in culture dishes (quality TC-60/15, Greiner, Nüreningen, FRG) whose bottom was covered with broken glass cover slips. After adherence of macrophages non-adherent cells were washed out. Cells were kept 5 to 20 days in an incubator (IR 1500 Automatic CO2 incubator Flow, Meckenheim, FRG) at 37°C and 5% CO2. Culture medium was exchanged twice a week.

Broken glass cover slips with adherent macrophages were positioned in the experimental bath (400 μl) which was placed on a movable stage of an inverted microscope (Nikon Inverted Microscope Diaphot-TMD, Düsseldorf, FRG). Bath electrolyte solution contained (in mmol/l) NaCl150, KCl 10, CaCl2 0.9, MgCl2 0.6, N-(2-hydroxyethyl)-piperaazine-N'-2-ethane-sulfonic acid (HEPES) 7.3, glucose 5. pH was adjusted to 7.2 with NaOH. The bath could be continuously perfused at a preselected rate, mostly 0.5 ml/min (pump: constructed and built by T. Weis). Macrophages were kept for a minimum of 15 min in the bath before recording started. Recordings lasted maximally 1 hour and were done at room temperature (mostly 23°C).

For whole-cell recordings (Hamill et al. 1981) a “patch clamp L/M-EP C 7” (List-Electronic, Darmstadt, FRG) was used. Micropipettes were produced out of filament-borosilicate glass (Hilgenberg, Malsfeld, FRG) by using a two-stage “patch electrode-puller” (Hans Ochotzki, Homburg, FRG) and filled with standard (intracellular) electrode solution which contained (in mmol/l): NaCl 10, KCl 140, CaCl2 1, MgCl2 1, HEPES 10, ethyleneglycol-bis-(β-aminoethyl ether)-NN,N,N',N'-tetraacetate acid (EGTA) 2.1. Offset between different electrode and indifferent bath electrode was compensated with the pipetric immersed in the bath solution. Diffusion potential was approximately ~4 mV (pipette negative) as compared to measurements where the pipette was dipped into intracellular pipette solution. This minor error was not corrected and thus transmembrane voltages are about ~4 mV more negative than indicated. Resistance of the different electrode was ≤10 MΩ. Seal resistance in the cell-attached mode was ≥10 GΩ. Patch membrane was ruptured by suction and change into the whole-cell recording mode was indicated by an about tenfold decrease of resistance and an enlarged capacitive current. If capacitative current was compensated at membrane potentials of ~40 mV and ~30 mV the EPC-7 dial gave readings in the range of 20 to 40 pF for most cells. Readings for series resistance were in the range of 10 to 20 MΩ. In uncompensated recordings maximal membrane current was ≤300 pA and according to conventional convention a maximal voltage error of 6 mV was calculated.

Voltage protocols were generated, recorded signals were digitized and numerical evaluation of recorded data was done with the aid of specialized hardware and software developed by G. Chini, D. Hafner and F.J. Katterbach (Institut für Pharmakologie, Universität Düsseldorf, FRG) for use in conjunction with a personal computer (Compaq 386 SX, München, FRG).

Averaged data were calculated as arithmetic means and standard errors of the mean (SEM). A Wilcoxon test was used for statistical evaluation. Mean values were considered to be significantly different with two tailed P-values of P<0.005.

Conductance voltage curves were constructed by using the equation g = A/Vm - Vref. Here g gives the conductance and A the current amplitude at Vm. Driving force is assumed to depend linearly on membrane potential and is given by the difference between test potential (Vm) and reversal potential of the current (Vref). Activation curves were fitted to the equation g/ gmax = 1/(1 + exp [(Vm - Vh)/K]) where gmax gives the maximal conductance, Vh gives the midpoint of the activation curve, K the slope factor and the other symbols are as defined above.

Mouse peritoneal macrophages were treated with human lipoproteins, a system which has been established in biochemical studies (Brown and Goldstein 1983). Effects on membrane currents mostly were recorded 5 to 10 min after starting of superfusion with lipoproteins. Low density lipoproteins (LDL) and acetylated low density lipoproteins (acetyl-LDL) had been kindly prepared by Prof. G. Schmitz and co-workers. Method of lipoprotein preparation is described elsewhere (Schmitz et al. 1987). Lipoprotein concentration is given in terms of protein content (μg/ml).

Results

Whole-cell currents under control conditions

Current-voltage curves of a single macrophage (Fig. 1) exhibit the main characteristics observed in most of the macrophages which had been maintained in culture for 5 up to 20 days prior to the experiments. Holding potential was about ~40 mV and 500 ms-lasting test pulses were delivered at a frequency of 0.2 Hz.

The amplitude of the rapidly activating outward current (iTo) which was measured at 10 to 20 ms after test pulse onset showed a steady increase in amplitude in the range from ~60 to +60 mV (Fig. 1A, B). Out of the channels described in macrophages this fastly activating outward current will flow through Ca-activated K-channels and/or Cl-channels (Gallin 1991). Inwardly rectifying K-channels are active at potentials negative to ~50 mV and will hardly be activated by the voltage protocol used (Gallin 1981; Gallin and Sheehy 1985; Ran-