THE EFFECT OF THE GLIAL CONDITIONED MEDIUM ON BRAIN AND SPINAL CORD MICROGLIAL CELLS IN CULTURE

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Abstract. Glial cells, in particular microglia, are important regulators of neuronal functions. Microglia are a heterogeneous population that react differently depending on the activating agents and on their location in the nervous system. Little is known about the action of the glial conditioned medium (GCM) on the membrane currents of these cells. Our study indicates that the GCM from a mixed glial culture has no effect on the resting membrane potential and is inducing a decreasing trend of the current density.

Key words: glial conditioned medium, microglial cells, electrophysiology, brain, spinal cord.

INTRODUCTION

Microglial cells represent one of the glial cells populations within the central nervous system (CNS). They are considered the circulating macrophages of the CNS, responsible for baseline surveillance, phagocytosis and apoptosis [9]. Microglia can be found in a resting state, characterized by a ramified cell body, or an activated state presenting an amoeboid shape [3]. Depending on their state, these cells express a large variety of receptors and ionic channels [5]. Even more, depending on the brain region where they originate, microglia can have different responses to activators [10] indicating an even higher heterogeneity.

The interaction between glial cells and neurons is well known [6], but little is known about how different mediators interfere with microglial ionic channels. At the spinal cord [14] and brain [13] levels, microglia react to different cytokines or chemokines released after an insult by an altered phenotype. In a similar fashion, the astrocyte conditioned medium (ACM) triggers a change in the brain microglia phenotype in culture, which becomes more ramified [4]. However, it is not clear yet how this conditioned medium is altering the membrane currents in the brain and spinal cord microglia. In this study we showed that the GCM from a mixed

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glial culture has no effect on the resting membrane potential, but it has a potential capacity to influence the current density.

MATERIALS AND METHODS

CELL CULTURE

All the experiments were carried out in accordance with the EU Directive 2010/63/EU for animal experiments and the ethical guidelines of the University of Bucharest. For this study we used brain and spinal cord from Wistar rat pups, P0–P1 to prepare mixed glial cultures. The tissue was minced with a blade and triturated in Dulbecco's modified Eagle's medium (DMEM, GIBCO). After centrifugation at 1500 rpm for 5 min, the pellet was re-suspended with culture medium, containing DMEM, 10% FBS (fetal bovine serum, GIBCO) and 1% P/S (penicillin/streptomycin, Sigma), then plated on 75 cm^2 flask. Individual flasks were used for brain and spinal cord tissue with medium replaced every 3 days until ~ 80% confluence. At this confluence, the medium was replaced with fresh one and harvested at day 2 or day 7 and stored at 4 °C for no more than 1 week, to keep the components of the GCM in a stable form. At ~ 100% confluence, microglia were obtained from the flasks containing the mixed glia culture by shaking at 190 rpm for 2 h. The cell suspension was centrifuged at 1500 rpm for 5 min, re-suspended with culture medium and plated on Petri dishes containing culture medium or the GCM to have the two conditions, control and treated, respectively. All the experiments were performed after 24 h incubation at 37 °C, 5% CO_2 with culture medium for control conditions, with GCM of 2 days or conditioned medium of 7 days. The recordings on microglia cells were made the next day to avoid the changes in the state of the activation, due to the culturing process.

ELECTROPHYSIOLOGY

Whole-cell patch-clamp was used to measure the membrane currents of microglia. The extracellular solution contained (in mM): NaCl 140, KCl 4, CaCl2 2, MgCl2 1, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) 10, adjusted with NaOH at pH 7.4. The intracellular solution contained (in mM): NaCl 5, KCl 130, CaCl2 1, MgCl2 2, HEPES 10, ethylene glycol-bis(2-aminoethylether)-N,N,N′,N′-tetraacetic acid (EGTA) 10, adjusted with KOH at pH 7.4. Patch pipettes were made from borosilicate glass (Harvard Apparatus, UK) and pulled at a final resistance of 4–5 MΩ, with a vertical puller (WPI, Berlin, Germany). The signal was amplified using a WPC-100 amplifier, digitized with DigiData 1322 and recorded with pClamp 8.1 software (Molecular Devices, USA).

Seal parameters were used as eliminatory criteria. All the reagents were from Sigma if not mentioned otherwise.

Current clamp recordings were made to analyze the resting membrane potential and voltage clamp recordings were made using voltage steps between –160 to +40 mV, from a holding potential of –60 mV. The membrane capacitance (C_m) was displayed by the recording software and used for the calculation of the current density using the formula: current density (pA/pF) = current (pA) / C_m (pF).

STATISTICS

The offline analysis was made using Clampfit 8.1 (Molecular Devices, USA), Microsoft Excel (Microsoft, USA) and Origin 8.5 (OriginLab Corporation, USA). For statistical analysis we used the Student's t-test, the values are presented as mean ± SEM and a value of $P < 0.05$ was considered significant.

RESULTS

The resting membrane potential of brain microglia in control conditions (–62.19 ± 8.07 mV, cells = 7, cultures = 2) was not significantly different after 24h treatment with the 2 days GCM (–51.32 ± 9.59 mV, cells = 5, cultures = 2, $P > 0.05$) or 7 days GCM (–48.07 ± 7.04 mV, cells = 5, cultures = 2, $P > 0.05$) (Fig. 1A). Similarly, the resting membrane potential of microglia from the spinal cord in control conditions (–47.49 ± 11.46 mV, cells = 6, cultures = 2), was not significantly different from that recorded in cells incubated for 24 h with the GCM of 2 days (–47.65 ± 8.47 mV, cells = 4, cultures = 2, $P > 0.05$) or 7 days (–39.30 ± 13.95 mV, cells = 4, cultures = 2, $P > 0.05$).

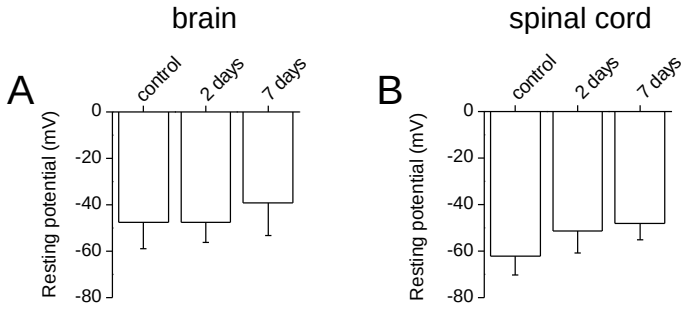

Fig. 1. The resting membrane potential in cultured microglial cells from the brain (A) and spinal cord (B).

The voltage-clamp recordings in brain microglia cells indicated a decreasing trend of the current density at negative potentials in cells treated for 24 h with GCM of 2 days (–4.72 ± 2.43 pA/pF, cells = 4, cultures = 2) or 7 days (–3.54 ± 0.92 pA/pF, cells = 5, cultures = 2) compared to control (–9.55 ± 3.19 pA/pF, cells = 6, cultures = = 2), even though the statistical significance was not reached at the peak of the current (P = 0.13). At positive potentials on the other hand, there was no current density difference in the three conditions (control 2.96 ± 0.89 pA/pF, cells = 6, cultures = 2; GCM of 2 days 1.49 ± 0.49 pA/pF, cells = 4, cultures = 2, $P > 0.05$; GCM of 7 days 2.04 ± 0.37 pA/pF, cells = 5, cultures = 2, $P > 0.05$) (Fig. 2A and C).

Spinal cord microglia in primary culture behaved differently in terms of the response to the GCM. At negative potentials, microglia incubated for 24 h with the GCM of 2 days (–3.60 ± 0.99 pA/pF, cells = 4, cultures = 2, $P > 0.05$) presented approximately the same current density as in control conditions (–4.45 ± 1.66 pA/pF, cells = 6, cultures = 2), while microglia treated for 24 with the GCM of 7 days showed a decreasing trend (–9.97 ± 4.08 pA/pF, cells = 4, cultures = 2, $P >$ 0.05). At positive potentials, microglia treated for 24 h with the GCM of 2 days presented almost the same current density as control cells (for control, 2.92 ± 0.47 pA/pF, cells = 6, cultures = 2; GCM of 2 days = 2.94 ± 0.86 pA/pF, cells = 4, cultures = 2, $P > 0.05$), while in cells incubated for 24 h with the GCM of 7 days (1.83 ± 0.39, cells = 4, cultures = 2, $P > 0.05$) the current density was slightly reduced (Fig. 2B and D).

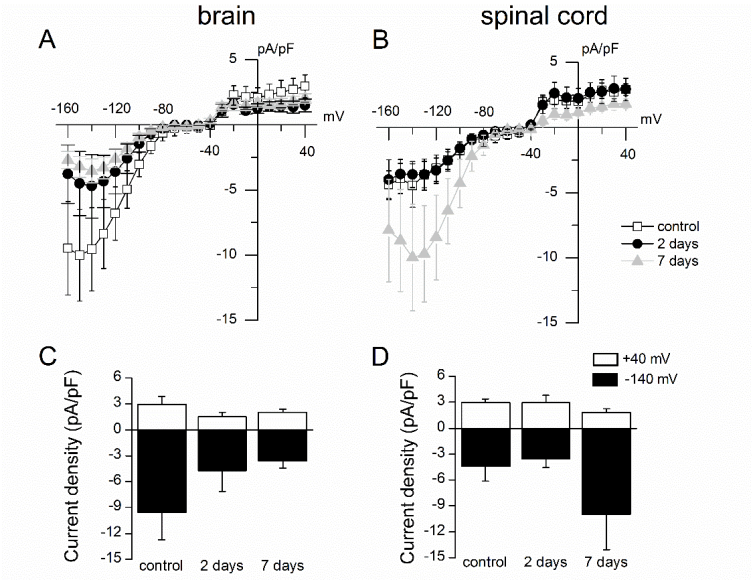

Fig. 2. The current density in cultured microglial cells from the brain and spinal cord. (A and B) I-V curves indicating the current density at each voltage step, between –160 mV to +40 mV. (C and D) Graph bars showing the statistical analysis of the current density at steps –140 mV and +40 mV.

DISCUSSION

Our study indicates that the GCM did not alter the resting membrane potential of microglial cells in culture, although it is inducing a change in the current density. Microglia, the resident macrophage of the central nervous system, behave differently, depending on their environment [8]. Microglia present a slightly more negative resting membrane potential when cultured compared with slices, due to the changes induced by the culturing process [2]. It was shown that microglial cells from aged mice displayed a more negative resting potential and an increased inward and outward rectifier potassium currents compared with young animals [12]. Microglia from the facial nucleus displayed a more negative resting potential and inward currents after the axotomy of the facial nerve [1]. After the disturbance of tissue homeostasis or experimental stimulation, microglia can shift their activity state and display a reactive profile [7]. Microglia phenotype changes during inflammation and brain tumor due to the secretion of cytokines, signaling molecules and growth factors [11]. The astrocyte conditioned medium contains different proteins that can change the activity of surrounding cells [15]. These studies indicate that the medium surrounding microglia cells can alter the expression of some ionic channels and therefore their electrical properties. Nevertheless, the GCM used in this study failed to significantly change the resting membrane potential in microglia from the brain and spinal cord, probably due to low levels of signaling molecules. Even if the differences against the control are not statistically significant the tendency showed by average values with the conditioning period suggests a potential capacity of the GCM to influence the current densities.

As previously described, the astrocyte conditioned medium did not change the current density of brain microglia cells [4]. In our study, we observed a decreasing trend due to the GCM at hyperpolarized and depolarized steps. In the brain there is a heterogeneous population of microglia which reacts differently to activating agents depending on their region [10]. Therefore, we expected that spinal cord microglia and brain microglia would behave differently in response to the GCM. Our data support this hypothesis, indicating a different trend in current density at negative and positive potentials depending on the origin of microglia.

CONCLUSION

Treatment of microglia with the GCM showed no effect on resting membrane potential irrespective if they come from brain or spinal cord, but it showed a potential significant effect on current density at negative potentials.

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