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Differential regulation of voltage-gated Ca^{2+} currents and metabotropic glutamate receptor activity by measles virus infection in rat cortical neurons

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ABSTRACT

Measles virus (MV) infection may lead to severe chronic CNS disease processes, including MV-induced encephalitis. Because the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) is a major determinant of the (patho-) physiological state in all cells we asked whether important Ca^{2+} conducting pathways are affected by MV infection in cultured cortical rat neurons. Patch-clamp measurements revealed a decrease in voltage-gated Ca^{2+} currents during MV-infection, while voltage-gated K^+ currents and NMDA-evoked currents were unaffected. Calcium-imaging experiments using 50 mM extracellular KCl showed reduced $[Ca^{2+}]_i$ increases in MV-infected neurons, confirming a decreased activity of voltage-gated Ca^{2+} channels. In contrast, the group-I metabotropic glutamate receptor (mGluR) agonist DHPG evoked changes in $[Ca^{2+}]_i$ that were increased in MV-infected cells. Our results show that MV infection conversely regulates Ca^{2+} signals induced by group-I mGluRs and by voltage-gated Ca^{2+} channels, suggesting that these physiological impairments may contribute to an altered function of cortical neurons during MV-induced encephalitis.

Measles virus (MV) belongs to the family of paramyxoviridae and is a member of the genus morbillivirus that primarily spreads via the respiratory system and replicates in endothelial cells, lymphocytes, monocytes and macrophages. However, the invasion of MV into the CNS seems to be a common event as it is reflected by a conspicuous electroencephalogram in up to 50% of the MV patients and by the finding of genomic sequences in normal autopsy cases [20]. The infection of the CNS can lead to severe complications, namely acute postinfectious encephalitis, measles inclusion body encephalitis, and subacute sclerosing panencephalitis [14,19]. The subacute sclerosing panencephalitis is a progressive neurodegenerative disorder due to inflammatory processes [14,20]. In the course of a chronic CNS inflammation, substances released from activated lymphocytes, macrophages and glial cells, such as NO and glutamate, can affect neurons and their physiological state. It has also been discussed that an altered distribution and expression of ion channels and transporters leading to increased neuronal excitability may contribute to the neurological symptoms in inflammatory neurodegeneration [23]. A number of viral infections have been shown to influence the activity of ion channels and neurotransmitter receptors in different cell types. Particularly, a reduced activity of voltage-gated calcium channels (VGCCs) has been described in Mumps virus-infected [26] as well as in influenza A virus-infected cultures of rat hippocampal neurons [6]. Down-regulation of VGCC mRNA was found in patients suffering from human immunodeficiency virus type-1 associated dementia [15]. VGCCs and ionotropic glutamate receptors provide major physiological Ca^{2+} entry mechanisms in neurons but also contribute to glutamate excitotoxicity and neurodegeneration. However, stimulation of metabotropic glutamate receptors (mGluRs) was shown to cause also neuroprotective effects [10], including the reduction of apoptosis markers [1,29] and the down-regulation of VGCC activity [9,22].

To study the role of MV-infection in neuronal Ca^{2+} entry mechanisms, that may have different effects on cell degeneration, the activity of VGCCs, *N*-methyl-D-aspartate receptors (NMDARs) and mGluRs in cultured cortical neurons was investigated.

Neuronal cultures were obtained from cortical tissue of Lewis rats at embryonic day 18. Cells were plated at a density of 1.5×10^5 cells/ml onto poly-D-lysine coated glass coverslips and cultured in Neurobasal medium (Gibco) supplemented with 2% B-27 (Gibco), 0.5 mM glutamine and 25 μM glutamic acid. MV infection with the Edmonston Zagreb strain or MOCK-infection was performed in Neurobasal medium without supplements for 1 h at 37 °C. After incubation the medium was replaced for Neurobasal medium supplemented with 2% B27 and 0.5 mM glutamine. Cultures ($n = 7$ independent cultures) for patch-clamp experiments were infected with a multiplicity of infection (m.o.i.) of 0.1 or 1.0 after 5 days *in vitro* (DIV). Cultures ($n = 5$ independent cultures) for

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calcium imaging experiments were infected with a m.o.i. of 1.0 after 4 DIV. Immunocytochemical staining of infected and non-infected cells was performed as described previously [13]. The anti-MV antibody mAb L77 (anti-haemagglutinin) was produced from hybridomas as described previously [21]. Cells were stained with fluorescein isothiocyanate (FITC)-conjugated secondary antibody [21].

Whole-cell Ca^{2+} and K^+ currents were recorded using the patch-clamp technique. Ca^{2+} currents were evoked by depolarizing test pulses (60 ms duration) from −50 mV to +60 mV (10 mV increment) from a holding potential of −60 mV. K^+ outward currents were evoked by test pulses (50 ms duration) from −40 to +100 mV (10 mV increment) from a holding potential of −60 mV. The bath solution for measurement of Ca^{2+} currents contained (in mM): choline-Cl, 130; TEA-Cl, 7.5; $CaCl_2$, 10; glucose, 10; HEPES, 10; buffered to pH 7.4. The corresponding pipette solution contained (in mM): CsCl, 120; TEA-Cl, 20; $CaCl_2$, 1; EGTA, 11; $MgCl_2$, 2; Mg-ATP, 5; HEPES, 10; buffered to pH 7.2. For recording K^+ outward currents, the bath solution had the following composition (in mM): NaCl, 140; KCl, 5; $CaCl_2$, 1.8; $MgCl_2$, 1; $CdCl_2$, 0.1; glucose, 10; HEPES, 10; buffered to pH 7.4. The corresponding pipette solution contained (in mM): KCl, 140; NaCl, 4; $CaCl_2$, 0.5; EGTA, 2; $MgCl_2$, 1; Mg-ATP, 5; HEPES, 10; buffered to pH 7.2. To suppress voltage-gated Na^+ currents both of the external bath solutions contained 1.5 μM tetrodotoxin (TTX). For data acquisition, an EPC-9 amplifier and pulse software (HEKA, Lambrecht, Germany) was used. Patch pipettes (4–6 MΩ) were filled with the respective internal solution. The mean input resistance was 17.5 ± 0.5 MΩ and 16.4 ± 0.5 MΩ for infected cells (n = 120) and non-infected cells (n = 100), respectively. Capacitive currents were corrected automatically. NMDA-induced currents were recorded using an external solution composed of (in mM): NaCl, 162; KCl 2.4; $CaCl_2$, 1.2; HEPES, 10; glucose, 10; glycine, 0.01; buffered to pH 7.4. Picrotoxin (100 μM), strychnine (2 μM) and TTX (1.5 μM) were added to suppress GABAergic, glycinergic and voltage-gated Na^+ currents, respectively. The corresponding pipette solution contained (in mM) CsCl, 140; $MgCl_2$, 2; $CaCl_2$, 1; EGTA, 11; HEPES, 10; buffered to pH 7.2. NMDA was applied onto single cells using a gravity-driven multi-channel application system.

The $[Ca^{2+}]_i$ was measured and calibrated as previously described [5]. Briefly, cells were loaded with 5 μM fura-2-AM (Molecular Probes) supplemented with 0.01% Pluronic F127 for 35 min at 20–22 °C in a bath solution containing (mM): NaCl, 140; KCl, 5; $MgCl_2$, 1; $CaCl_2$, 2; glucose 10; HEPES, 10; adjusted to pH 7.4. For measurements of $[Ca^{2+}]_i$, cells were kept in the same bath solution and fluorescence was excited at 340 and 380 nm using a monochromator-based imaging system (T.I.L.L. Photonics, Gräfelfing, Germany) attached to an inverted microscope (BX51WI, Olympus, Hamburg, Germany). Emitted fluorescence from single cells was collected by a charge-coupled device camera at intervals of 2 s.

All chemicals were purchased from Sigma–Aldrich. Data are derived from individual cells and are expressed as mean ± S.E.M. Imaging experiments were performed four times (application of NMDA) or six times (application of KCl and DHPG) for every condition. Statistical significance ($^*p < 0.05$ or $^{***}p < 0.001$) between infected and non-infected cells was determined by unpaired Student's t-test.

To verify the presence of MV in neuronal cultures we used a monoclonal antibody (mAb L77) against the MV haemagglutinin envelope protein. MV-haemagglutinin was detectable 1–10 days post infection (DPI). About 60–70% of cells showed MV-infection at 5 DPI (Fig. 1A, B) irrespective of the m.o.i. (0.1 or 1.0).

First, we tested whether MV-infection affects voltage-gated Ca^{2+} currents in cultured cortical neurons at 10–12 DIV, corresponding to 5–7 DPI. Ca^{2+} currents were isolated from other voltage-dependent membrane currents by ionic composition of both the pipette and bath solution as well as addition of TTX to the bath solution. Cultured cortical neurons predominantly express high voltage-activated (HVA) slowly inactivating Ca^{2+} channels in conjunction with neurite outgrowth at later culture stages [30]. Ca^{2+} currents in MV-infected as well as non-infected cortical neurons evoked by depolarizing test pulses from a holding potential of −60 mV showed characteristics of HVA Ca^{2+} channels (Fig. 2A). Peak Ca^{2+} inward currents attained a maximum at test potentials between +10 and +20 mV (Fig. 2B). To test for a possible change in other ion conductances we analysed the functional expression of voltage-gated K^+ channels and of NMDA receptors in MV-infected and non-infected cells from the same culture stage. K^+ outward currents were evoked by depolarising voltage steps from a holding potential of −60 mV (Fig. 2C) and showed a slow inactivation similar to those observed in cultured hippocampal neurons [12]. NMDAR-mediated currents were evoked by rapid application of 30 μM NMDA from a holding potential of −70 mV (Fig. 2D). From the peak inward and outward currents of NMDARs, VGCCs, voltage-gated K^+ channels, and the corresponding

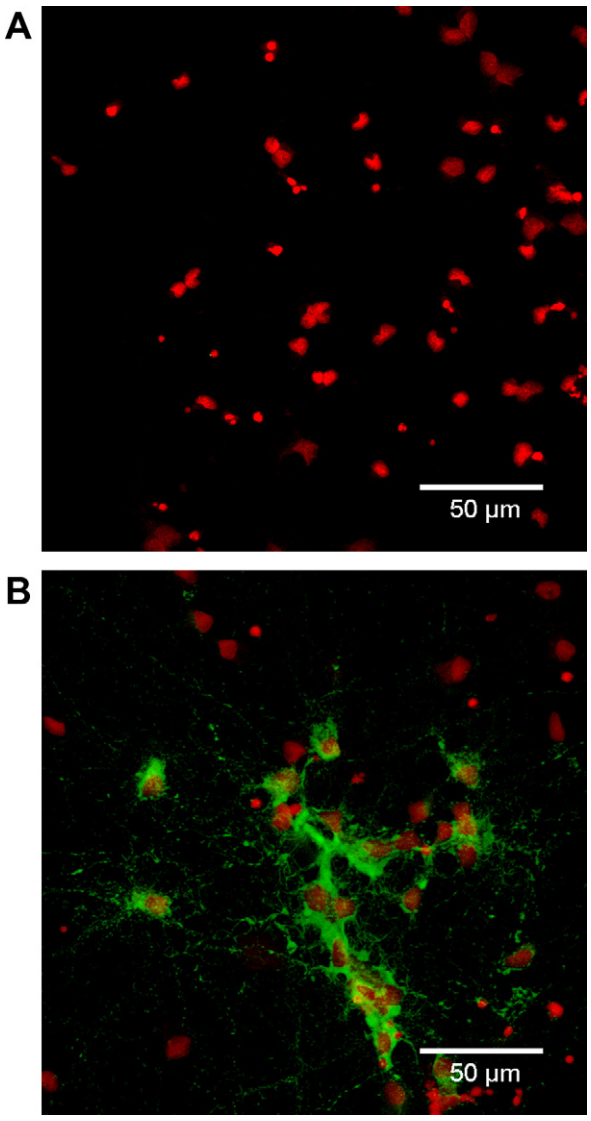

Fig. 1. Immunocytochemistry of cultured cortical neurons after 10 days *in vitro* (DIV) cultivation. Non-infected neurons (A) and MV-infected neurons (B) 5 days *post infection* (DPI) were stained with a monoclonal antibody against MV-haemagglutinin protein (green) and counterstained with propidium iodide (red). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)