Brain Research Bulletin 117 (2015) 54–68

Contents lists available at ScienceDirect

Brain Research Bulletin

journal homepage: www.elsevier.com/locate/brainresbull

Research report

Suppression of excitotoxicity and foreign body response by memantine in chronic cannula implantation into the rat brain

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ARTICLE INFO

Article history:
Received 22 June 2015
Received in revised form 30 July 2015
Accepted 3 August 2015
Available online 6 August 2015

Keywords:
Excitotoxicity
Foreign body response
Glutamate
Implant
Memantine
Skilled reaching task

ABSTRACT

Chronic brain implants are accompanied by a tissue response that causes the loss of neurons in the vicinity of the implant and the formation of a glial scar that is also referred to as foreign body response. Despite immense progress in the field of brain-computer interface (BCI) research the biocompatibility of chronic brain implants remains a primary concern in device design. Excitotoxic overstimulation of NMDA-receptors by extrasynaptic glutamate plays a pivotal role in cell death in the acute phase of the tissue reaction. In this study, we examined the effect of the uncompetitive NMDA-receptor antagonist memantine locally applied during cannula implantation in the caudal forelimb area (CFA) of the motor cortex (M1) in Lister Hooded rats on their behavioural performance in a skilled reaching and a rung-ladder task as well as in the open field. Moreover, the distribution of neurons and glial cells in the vicinity of the implant were assessed. Memantine improved the performance in the behavioural paradigms compared to controls and increased the number of surviving neurons in the vicinity of the implant even above basal levels accompanied by a reduction in astrocytic scar formation directly around the implant with no effect on the microglia/macrophage activation two and six weeks after cannula implantation. These findings suggest that memantine is a potential therapeutic agent in the acute phase of chronic foreign body implantation in the motor cortex in terms of increasing the viability of neurons adjacent to the implant and of improving the behavioural outcome after surgery.

1. Introduction

In the last decades immense progress has been made in the field of brain-computer interface (BCI) research. Neuroprosthetic devices that restore mobility in patients suffering from paralysis or amputations have been developed, and moreover, have demonstrated functionality even years after an injury to the central nervous system (CNS) (Hochberg et al., 2012; Jackson, 2012). Despite this technological progress, the biocompatibility of these chronic brain implants still remains a major problem and a demanding field of research. Different electrode designs as well as implantation techniques have been investigated (Edell et al., 1992; Turner et al., 1999; Szarowski et al., 2003; Nicolelis et al., 2003; Kim et al., 2004; Biran et al., 2005), complemented by different surface coatings with biocompatible (Ignatius et al., 1998; Kam et al., 2002; He et al., 2006) or immunosuppressant molecules (Maynard et al., 2000; Shain et al., 2003; Kim and Martin, 2006; He et al., 2007). However, a satisfactory control of the foreign body reaction that may impair the functioning of the implants is still not available (Polikov et al., 2005; Griffith and Humphrey, 2006).

The implantation into the brain of a chronically indwelling device, such as an electrode array, is accompanied by two different phases of tissue reaction: an acute and a chronic response (Turner et al., 1999; Szarowski et al., 2003). The acute response is caused by the rupture of blood vessels in the implantation tract and the damage of neurons and glial cells (Szarowski et al., 2003; Potter et al., 2012). Similar to stroke, the disruption of blood vessels in the implantation tract impairs the oxygen and energy supply of neurons which causes an ionic imbalance that is followed by sustained release of glutamate due to uncontrolled membrane depolarisation (Iadecola and Anrather, 2011; Bretón and Rodríguez, 2012). Glutamate is suggested to be either calcium-dependently released from neuronal vesicles, potassium-dependently through swelling-activated anion channels from astrocytes, or sodium-dependently by a reversed operation of glutamate transporters (Rossi et al., 2000). The accumulation of glutamate in the extracellular space

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http://dx.doi.org/10.1016/j.brainresbull.2015.08.001
0361-9230/

overstimulates glutamate receptors with *N*-methyl-*D*-aspartate (NMDA) receptors playing a pivotal role in excitotoxic cell death (Lipton and Rosenberg, 1994; Lynch and Guttmann, 2002). The two major subtypes of NMDA receptors in the adult forebrain are the NR2A-subunit containing receptor that is primarily located at the synapse activating intracellular processes resulting in neuronal survival, and the NR2B-subunit containing receptor that is primarily located at extrasynaptic sites activating downstream cascades resulting in neuronal death (Hardingham et al., 2002; Lai et al., 2011; Vizi et al., 2013). Excessive Ca^{2+} influx into the cells via extracellular NR2B-receptors induces an overproduction of highly reactive free radicals, mitochondrial dysfunction, cell membrane rupture and DNA fragmentation, which results in excitotoxic cell death of apoptotic or necrotic nature depending on the severity of the insult (Bretón and Rodríguez, 2012). Moreover, the injury of neurons causes further leakage of excitotoxic amounts of the amino acid glutamate, enhancing the cascades of apoptotic and necrotic processes by NMDA receptor overstimulation. The large amounts of glutamate spill over to nearby cells that had survived the original trauma and cause them to depolarise, swell, lyse and to release their cellular contents into the parenchyma. This autodestructive cascade continues for hours or even days (Bonfoco et al., 1995; Lipton, 2006). The necrotic brain tissue and the accumulation of fluid in the acute phase accompanied by the release of early neuronal 'danger signals' like the nucleotide ATP by the injured cells activates microglia cells that migrate to the site of injury in order to clear the necrotic tissue and to degrade the foreign body by releasing pro-inflammatory molecules (Davalos et al., 2005; Iadecola and Anrather, 2011). This in turn activates astrocytes that extend their processes towards the implantation site to separate the healthy tissue from the injured. These early reactive responses of the glial cells are supposed to be complete about two weeks after the implantation (Turner et al., 1999; Szarowski et al., 2003). In contrast to stab wounds though, these acute responses are followed by long-term inflammatory processes around the implant in chronic settings with microglia cells trying to degrade the foreign body by means of enduring release of inflammatory mediators (Biran et al., 2005; Potter et al., 2012). About six weeks after the implantation, the glial scar is highly compact and remains constant in size after this time point to isolate the healthy brain tissue from the enduring inflammatory processes at the interface (Turner et al., 1999; Szarowski et al., 2003). For optimal device function of brain electrodes the survival of neurons within the first 50 μm around the implant is of special importance (Buzsaki, 2004). However, device implantation followed by the neurotoxic cascade and the enduring inflammation processes, results in a neuronal "kill zone" around the implantation site (Edell et al., 1992; Biran et al., 2005), followed by the formation of a glial scar that pushes the surviving neurons even further away. Moreover, the glial scar prevents the regrowth of neuronal processes into the implantation site, since this process takes longer than sheath formation itself (Turner et al., 1999).

The current study serves as a proof-of-principle investigation mimicking the implantation of a device such as an electrode array into the motor cortex by means of a steel cannula. We aimed to block the initial steps of the foreign body response and to counteract the extent of neurotoxicity at the implantation site by a single local application of the uncompetitive NMDA receptor antagonist memantine. The relatively low-affinity, open-channel blocker memantine, is the first clinically successful NMDA receptor antagonist that is used for the treatment of Alzheimer's disease (Scarpini et al., 2003; Lipton, 2006; Wenk et al., 2006) and also shows some potential therapeutic benefit in patients with Huntington's disease (Fan and Raymond, 2007) with a more favourable side-effect profile than high affinity uncompetitive NMDA receptor antagonists (Parsons et al., 1999). The progressive cell death due to NMDA receptor overactivation gets inhibited by memantine without affecting the physiological functions at the synapse due to its fast off-rate kinetics (Parsons et al., 1999; Lipton, 2004) and its preference for extrasynaptically located NR2B-receptors over synaptically located NR2A-receptors (Xia et al., 2010). In the context of this study, memantine administration during cannula implantation may mimic the local drug delivery from coated electrodes and is expected to facilitate the survival of neurons in the vicinity of the implant and to suppress the density of the glial scar. We investigated the skilled reaching performance of rats based on the behavioural paradigm of Whishaw et al. (Whishaw et al., 1986; Whishaw and Pellis, 1990), the skilled walking performance on a rung ladder in different conditions (Metz and Whishaw 2002; Schlorke et al., 2013) and the locomotor behaviour of rats in an open field box before and after the implantation of a cannula in the caudal forelimb area (CFA) of the motor cortex (M1). Moreover, the spatial distribution of neurons, and glial cells was immunohistologically analyzed at the implantation site two weeks after cannula implantation when the glial sheath is supposed to be less organized and extends furthest around the implantation site (acute phase), and six weeks after implantation when the glial sheath is most densely organized and remains constant for the duration of the implantation (chronic phase) (Polikov et al., 2005).

2. Materials and methods

2.1. Animals

A total of 60 naive adult male Lister Hooded rats (Charles River Laboratories, Germany) weighing 200–220 g were used in this study. Rats were group-housed under standard conditions in Makrolon cages (type IV) on a 12 h light/dark cycle (lights on at 7:00 a.m.) with controlled temperature and humidity. All rats received tap water ad libitum and were restricted to 12 g standard diet rodent chow (Altromin, Germany) per rat per day as soon as the training started. The experiments were conducted in compliance with the ethical guidelines of the National Institute of Health for the care and use of laboratory animals for experiments and were approved by the local animal care committee (Senatorische Behörde, Bremen, Germany).

2.2. Timeline

The study was designed to assess the effects of acute memantine treatment locally administered during the implantation of a foreign body in the CFA of M1. Therefore, the rats were habituated and individually trained in the single-pellet boxes (Whishaw et al., 1986; Whishaw and Pellis, 1990) at least four weeks before the experiments started. After reaching a stable baseline of at least 75% the rats were pseudo-randomly assigned to six groups, receiving either artificial cerebrospinal fluid (aCSF) which served as the control or memantine in a low dose (20 μg/μl) or in a high dose (50 μg/μl). Brains were removed for histological analysis either two or six weeks after cannula implantation. All animals were tested in the single-pellet reaching box and in the open field the day before cannula implantation and once a week after the implantation. Moreover, the 2-week group was tested on a ladder rung walking task for further analysis of the rats' skilled walking ability and received one week of previous training.

2.3. Single-pellet reaching task

The single-pellet reaching task was chosen to define the preferred forepaw of each rat and to analyse the functionality of the motor cortex contralateral to this paw before and after cannula implantation.