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Research Report

Effects of chronic Clozapine administration on apolipoprotein D levels and on functional recovery following experimental stroke

Karsten Ruscher, Agnes Erickson, Enida Kuric, Ana R. Inácio, Tadeusz Wieloch*

Laboratory for Experimental Brain Research, Wallenberg Neuroscience Center, University of Lund, BMC A13, S-22184 Lund, Sweden

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ABSTRACT

Elevated brain levels of apolipoprotein D (ApoD) correlate with improved neurological recovery after experimental stroke. Hence, a pharmacological induction of ApoD in the postischemic brain could be beneficial for recovery after stroke. Here we investigated the effect of Clozapine, a compound that increases the expression of ApoD, in two rat models of experimental stroke. Rats were subjected to permanent occlusion of the middle cerebral artery (pMCAO) and treated with Clozapine (i.p. 10 mg/kg body weight) or saline for 8 or 28 days starting on the second day after MCAO. ApoD levels increased by 35% in the peri-infarct area after 10 and 30 days after pMCAO, mainly in neuron-specific nuclear protein (NeuN) positive neurons and glial fibrillary acidic protein (GFAP) positive astrocytes. Clozapine did not affect the neurological deficit assessed by the rotating pole test and a grip strength test at 7 days, 14 days, 21 days, and 28 days after pMCAO. Functional outcome and the infarct size were similar in rats subjected to transient MCAO and injected with Clozapine (i.p. 10 mg/kg body weight) or saline for 26 days starting on the second day after tMCAO. We conclude that Clozapine affects cellular processes involved in peri-infarct tissue reorganization, but does not affect functional recovery after MCAO.

1. Introduction

Mechanisms of brain reorganization, plasticity and repair promote spontaneous recovery in experimental models of stroke and in patients (Cramer, 2008). Enhanced functional recovery after experimental stroke correlates with the expression of growth associated proteins (Schneider et al., 2005), the regulation of lipid transport molecules (Rickhag et al., 2008) but also cell migration and differentiation (Komitova et al., 2006). It has further been shown that changes in dendritic arborization and spinogenesis, but also a long-term activation of ionotropic glutamate receptors may contribute to activation of new neuronal circuits (Dancause et al., 2005).

In an experimental paradigm where rodents subjected to middle cerebral artery occlusion (MCAO) are either housed in standard cages or housed in an enriched environment, the latter housing condition consistently stimulates neurological recovery (Ohlsson and Johansson, 1995). Importantly, recovery occurs without affecting infarct size. Based on gene array data (Rickhag et al., 2006), we have earlier shown that the lipocalin ApoD is upregulated in matured oligodendrocytes and reactive astrocytes in the peri-infarct area after experimental

* *Corresponding author*. Fax: +46 46 2220615.
E-mail address: karsten.ruscher@med.lu.se (K. Ruscher).

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stroke. Importantly, elevated levels of ApoD are associated with an improved outcome of rats housed in an enriched environment following stroke (Rickhag et al., 2008). In other pathological conditions, ApoD has been shown to accumulate at sites of regenerating peripheral nerves (Boyles et al., 1990) and in the cerebrospinal fluid of patients with neurodegenerative disorders, such as Alzheimer's disease or Niemann–Pick's type C disease (Terrisse et al., 1998; Suresh et al., 1998; Thomas et al., 2003a,b). ApoD may, therefore, participate in regeneration of the central and peripheral neurons by stimulation of neurite outgrowth and expression of pre- and post-synaptic proteins (Kosacka et al., 2009).

Levels of ApoD are altered in the blood and elevated in brains of patients with schizophrenia and further elevated by Clozapine, an atypical neuroleptic used in treatment of the disorder (Thomas et al., 2001b; 2003a,b; Mahadik et al., 2002). Elevated levels of ApoD were also observed in the rodent brain after Clozapine treatment (Thomas et al., 2001a; Khan et al., 2002). Clozapine has effectively been shown to modulate ApoD mediated reduction of fatty acids related to the metabolism of arachidonic acid (AA). Also, levels of arachidonic acid are elevated in mice lacking ApoD (Thomas and Yao, 2007). Since ApoD shows a high affinity for AA (Vogt and Skerra, 2001) it may be involved in inactivation of oxidated AA derivates and prevention of AA peroxidation to promote membrane stability (Khan, et al., 2002; Yao et al., 2005). Clozapine could therefore be an interesting tool to pharmacologically induce ApoD during pathological conditions.

Developing molecular tools that modulate cellular and molecular processes promoting stroke recovery, particularly those occurring in the peri-infarct area (Carmichael, 2008), is a central theme in the search for new rehabilitative post-stroke therapies. The present study therefore was designed to evaluate if chronic Clozapine administration increases the level of ApoD in the peri-infarct area during the first 30 days of recovery and if this is accompanied by an improvement of neurological recovery after experimental stroke.

2. Results

2.1. *Chronic Clozapine administration increases ApoD levels in the peri-infarct area after pMCAO*

Treatment with Clozapine increases ApoD levels in the rodent and human brain (Thomas et al., 2001a). Here we injected rats with Clozapine (i.p. 10 mg/kg bw) or vehicle either for 8 or 28 consecutive days starting at day 2 after pMCAO. As shown in Fig. 1, ApoD levels were significantly elevated in the peri-infarct area of rats injected with Clozapine at 10 ($n=4$) and 30 days ($n=3$) of recovery, respectively. In contrast, ApoD levels were unchanged in the non-lesioned contralateral neocortex of rats after 30 days (Fig. 1). Taken together, our results corroborate previous findings showing an upregulation of ApoD in Clozapine treated animals.

We have shown that GST-π^+ oligodendrocytes of the peri-infarct area express ApoD (Rickhag et al., 2008). In the present study, ApoD immunoreactivity (ir) was found in matured oligodendrocytes of the proximal peri-infarct at 28 days after tMCAO. However, ApoD ir in oligodendrocytes did not differ between the two treatment regimes at this particular time point (Fig. 2A). In addition, no ApoD ir was detected in Iba1$^+$ microglia/macrophages (Fig. 2B). More interestingly, we found that NeuN$^+$ neurons located at the peri-infarct/infarct core border zone express ApoD in rats injected with Clozapine for 26 days after tMCAO (Fig. 3A). In contrast, vehicle treated animals showed only a weak ApoD ir in those neurons (Fig. 3B).

Major differences in ApoD expression between the treatment regimes were also observed in GFAP$^+$ astrocytes of the ischemic hemisphere. While GFAP$^+$ astrocytes form a sufficient peri-infarct scar to encapsulate the ischemic core in vehicle treated animals, we found a massive accumulation of GFAP$^+$ astrocytes throughout the ischemic hemisphere of Clozapine treated rats (Fig. 4). In addition, those astrocytes showed a strong ApoD ir contributing to the increase of ApoD levels (Fig. 1) in the peri-infarct area of Clozapine treated rats after MCAO.

2.2. *Clozapine did not affect functional recovery after MCAO*

We earlier found a strong correlation between increased ApoD levels in matured oligodendrocytes of the peri-infarct area and an improved recovery in rats housed in an enriched environment after experimental stroke (Rickhag et al., 2008). To investigate if Clozapine affects functional recovery after pMCAO, the neurological deficit of rats treated with Clozapine (cloz, $n=7$) or vehicle (vh, $n=8$) was assessed with the rotating pole test, a sensitive approach to test sensori-motor function and coordination. Forty-eight hours after pMCAO and prior to the first injection, rats of both groups had a rotating pole score of 0, i.e. they fell off the pole immediately (Fig. 5). In the following time course, rats showed partial recovery and were able to traverse the pole at 7 days after pMCAO. However, no differences were observed between the treatment groups, though Clozapine treated rats had a slightly better recovery at 14 days following pMCAO. Thirty days after pMCAO, rats were fully recovered to the same extent in both treatment groups. During the study, body weight was monitored daily and showed no differences between the treatment groups (day 14: cloz 338 g, 25th percentile 321 g, 75th percentile 340 g; vh 348.5 g 25th percentile 330.5 g, 75th percentile 357.5 g; day 30: cloz 340 g, 25th percentile 334 g, 75th percentile 348 g; vh 351 g 25th percentile 336 g, 75th percentile 370.5 g) indicating that Clozapine did not exert metabolic disturbances as described previously (Yazici et al., 1998).

In a model of experimental stroke with reperfusion rats were subjected to transient MCAO for 120 min. In summary, we could not detect a significant difference in the two behavioral tests employed. Using the rotating pole test we found a similar rate of spontaneous recovery in vehicle and Clozapine treated rats at 2, 7, 14, and 28 days of recovery (Fig. 6). We also used a standardized grip test to measure the maximum strength of the paralyzed (left) and non-paralyzed (right) front paw before and after MCAO. As shown in Fig. 7, transient occlusion of the middle cerebral artery provoked a substantial deficit in the corresponding left paw determined by measuring the grip strength of the paralyzed forelimb. However, no differences were observed between Clozapine and vehicle treated rats at the indicated time points following tMCAO. Also, chronic Clozapine treatment did not affect the performance of sham operated rats.