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Quinolinic acid-immunoreactivity in the naïve mouse brain

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ABSTRACT

Quinolinic acid (QUIN) has been suggested to be involved in infections, inflammatory neurological disorders and in the development of psychiatric disorders. In this view, several studies have been performed to investigate QUIN localization in the brain and its neurotoxic effects. However, evidence is lacking regarding QUIN in healthy, control conditions. The aim of this study was to investigate the region-specific distribution and pattern of QUIN expression in the naïve mouse brain. In addition, possible sex differences in QUIN-immunoreactivity and its link with affect-related behavioural observations were assessed.

For this purpose, naïve mice were subjected to the forced swim test (FST) and 20 min open field (OF) testing to measure affect-related behaviour. Afterwards, brains were assessed for QUIN-immunoreactivity. QUIN-immunoreactivity was particularly observed in the cingulate cortex (CC), highlighting clearly delineated cells, and the thalamic reticular nucleus (TRN), showing a more diffuse staining pattern. Subsequently, QUIN-positive cells in the CC were counted, while QUIN-immunoreactivity in the TRN was examined using gray value measurements. No significant differences between sexes were observed for the number of QUIN-positive cells in the CC, neither in levels of QUIN-immunoreactivity in the TRN. A direct correlation was found between QUIN-positive cells in the CC and QUIN-immunoreactivity in the TRN. Moreover, in male mice, a very strong correlation ($r_{sp} = .943$; $p < .01$) between QUIN-immunoreactivity at the level of the TRN and motor activity in the OF was observed. Thus, our results suggest that QUIN – detected in the CC and the TRN – may play a role in regulating motor activity in normal conditions.

1. Introduction

Quinolinic acid (QUIN) is an endogenous *N*-methyl-D-aspartate (NMDA) glutamate receptor agonist produced by the kynurenine pathway, particularly in the liver and the brain. The kynurenine pathway, which has been suggested to be implicated in the development of psychiatric disorders (Asp et al., 2011; Myint, 2012), involves the catabolism of tryptophan to kynurenine, which in turn can be further metabolized into the intermediates kynurenic acid (KYNA) or QUIN. Alterations in tryptophan, kynurenine, KYNA and QUIN have been reported in patients suffering from depression, schizophrenia and Alzheimer's disease, among others (Németh et al., 2006; Nilsson et al., in press; Gulaj et al., 2010; Myint et al., 2007). In depressive patients, increased cerebrospinal fluid (CSF) levels of QUIN have been observed (Raison et al., 2010), whereas another recent study showed decreased QUIN-immunoreactivity in the hippocampus of patients suffering from depression (Busse et al., 2015).

Evidence suggests that the production and accumulation of QUIN in the central nervous system (CNS) is implicated in several inflammatory neurological disorders and infections (Dang et al., 2000). It is well-known that during inflammation, cytokines are produced which mediate a subset of distinct inflammatory reactions (Kronfol and Remick, 2000; Zhang and An, 2007). Already in 1992, a study reported increased QUIN in lumbar CSF and post-mortem brain tissue of patients with inflammatory diseases, but not in CSF of patients with non-inflammatory neurological disease (Heyes et al., 1992). Moreover, in depression associated with a pro-inflammatory status, QUIN-immunoreactivity was found to be increased in the anterior midcingulate cortex

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(aMCC), the subgenual anterior cingulate cortex (sACC) and the cortical grey matter (Steiner et al., 2011). QUIN has been reported to aggravate the negative consequences of pro-inflammatory cytokines (Maes, 2008) and to exert various neurotoxic effects, inducing neuronal cell loss and convulsions (Nemeth et al., 2005). QUIN has also been linked to the induction of hippocampal cell death, selective necrosis of granule cells, destruction of postsynaptic elements and a reduction in cerebral cholinergic circuits (Maes et al., 2009).

In the CNS, QUIN is produced by activated microglia and macrophages. Different studies with discordant results have been conducted using several animal models and human tissues to determine the cellular localization of QUIN in the brain. In brain tissue from naïve control rats, no QUIN-immunoreactivity was observed (Moffett et al., 1993). However, different types of QUIN-positive cells, such as microglia, macrophage and ameboid-like cells were seen in the subiculum, dorsal hippocampus (CA1) and in the stratum pyramidale of the whole CA1 area in a gerbil model of ischemia (Baratte et al., 1998). In another study, high QUIN-immunoreactivity was observed only after focal injury of the neocortex in adult rats and was limited to those microglia directly within the lesion tract. In addition, QUIN-immunoreactivity was also seen in reactive cultured microglia and also in microglia of primary cultures of human mixed brain cells after IFN-γ stimulation (Chung et al., 2009; Guillemin et al., 2005a).

Despite the fact that research within this field has rapidly expanded in the context of pathological conditions, the normal, physiological role of QUIN is less clear. To date, no report showed the localization of QUIN and the pattern of its staining in the brain of naïve mice. Therefore, we analyzed QUIN-immunoreactivity in the naïve mouse brain. Our first objective was to examine the distribution and localization of QUIN-immunoreactivity. Second, we evaluated whether QUIN-immunoreactivity differs between sexes. Third and last, we investigated the possible association between QUIN-immunoreactivity and a subset of affect-related behavioural phenotypes.

2. Material and methods

2.1. Animals

C57BL/6JRccHsd mice were obtained from Harlan Laboratories (Eystrup, Germany). Mice were housed in single-sex groups of 4 mice per cage in individually ventilated cages (IVC 480 × 375 × 210 mm [depth × width × height]). Mice were kept under specific pathogen-free (SPF) conditions and maintained on a 12/12 h light-dark cycle (light on at 12 a.m.) and temperature-controlled environment (relative humidity 55% ± 5%; temperature: 22 °C ± 2 °C, room air exchange rate 15). Cages were changed every week. The habituation period lasted 3 weeks once the mice arrived to the local animal facilities. During this period, mice were subcutaneously injected with a transponder to facilitate identification. Breeding food (Ssniff, Germany) and water were allowed ad libitum. Experiments were performed according to the permissions from the government and the veterinarian administration of Oberbayern (AZ: 55.2-1-54-2531-61-10).

2.2. Behavioural tests

2.2.1. Forced swim test

At 12 weeks of age, mice were tested in the forced swim test (FST). This test consists of 4 vertical glass cylinders (height 57.5 cm × diameter 20 cm) filled to a depth of 50 cm with tap water at 22 °C. Cylinders were situated in front of a frosted glass screen and were illuminated by 4 infrared floodlights (Videor, Germany) behind the screen. Cylinders were separated by opaque partitions so that mice could not see each other. One animal was placed in each cylinder and tested for 6 min, between 12 p.m. and 5 p.m. After each test, water was changed and mice were dried and put back into their home cages. A wall-mounted camera (type EQ-150, EverFocus, Germany) connected to a converter (ADVC-55 Advanced DV Converter, Canopus GmbH, Germany) was used to record the test. Biobserve Forced Swim Test Version 1.5 software (BIObserve, Germany) was employed to analyse the following behaviours: (1) floating (absence of any movement or minor movements needed to keep the head above the water), (2) swimming (movements of the limbs associated with swimming and less severe than those associated with climbing) and (3) struggling (large movements associated with attempts to escape the swim tank).

2.2.2. Open field test

One week after the FST, mice were tested in the open field test (OF), consisting of a white PVC box (50 × 50 × 50 cm; Kümpel Kunststoffverarbeitungswerke GmbH, Germany). Two infrared floodlights (type WFL-II/LED30, Videor, Germany), mounted on tripods (type 3150, Cullmann, Germany) were situated on two sides of the OF area (containing 4 boxes). One animal was placed in each box and was allowed to explore the arena for 20 min. Testing was performed between 12 p.m. and 5 p.m. Boxes were cleaned after each measurement with water and alcohol and mice were returned to their cages. Behaviour was recorded with a camera (type AU-CB600, VIDO, Austria) connected to a converter (ADVC-55 Advanced DV Converter, canopus GmbH, Germany). The video-tracking software Viewer Version 3 (BIObserve, Germany) was used to score the time spent in each zone (s), track length (cm) and zone crossings (counts) in the whole box. Each box was divided as follows: the centre (34.6 × 34.6 cm; $W \times D$), 4 walls (each: 7 × 34.6 cm; $W \times D$) and 4 corners (each: 7 × 7 cm; $W \times D$).

2.3. Perfusion and extraction of organs

Thirteen week old mice were perfused 1 month after OF testing. Male [$n = 6$] and female [$n = 7$] mice were anesthetized with a single intraperitoneal injection of ketamin (Pfizer, Germany) and xylacin (Bayer, Germany). Subsequently, they were perfused with a heparinized saline solution followed by 4% paraformaldehyde (PFA) and 15% saturated picric acid. Brains were dissected and postfixed in 4% PFA overnight before being transferred to PBS-containing sodium azide (1%) and stored at 4 °C.

2.4. Immunohistochemistry

Thirty μm coronal brain sections of the brains were cut using a vibratome (Leica VT1200 S, Germany). Free-floating slices were rinsed for 3 times (Tris-buffered Saline with .3% Triton X-100 (TBS-T)/Tris-buffered Saline (TBS)/TBS-T; each for 10 min) and pre-incubated with 1% H_2O_2 in TBS to block endogenous peroxidase activity for 30 min at room temperature (RT). Tissue sections were washed again as mentioned above and incubated overnight at 4 °C with the primary polyclonal rabbit anti-QUIN antibody (1:1500 inTBS-T, apDia, Turnout, Belgium) (Gos et al., 2014). Next, sections were washed and incubated with biotinylated donkey anti-rabbit (1:400 in TBST-T, Jackson Laboratories, US) for 90 min at RT. Sections were washed again and tissue was further incubated with avidin-biotin complex (ABC) (1:400 in TBS-T, Vector Immunolabs) for 90 min at RT. After incubation, slices were washed twice with TBS for 10 min and once with .1 M acetate buffer (pH 6.0) for 15 min. Subsequently, tissue was incubated for 20 min at RT with glucose oxidase and DAB, and washed twice for 15 min with .1 M acetate buffer (pH 6.0). Finally, brain sections were mounted on coated microscope slides, dehydrated using an ethanol series,