Neuroscience 259 (2014) 63–70

CONGENITAL TAURINE DEFICIENCY IN MICE IS ASSOCIATED WITH REDUCED SENSITIVITY TO NOCICEPTIVE CHEMICAL STIMULATION

J. LÖTSCH,[a,b]* T. HUMMEL,[c] U. WARSKULAT,[d] O. COSTE,[a] D. HÄUSSINGER,[d] G. GEISSLINGER[a,b] AND I. TEGEDER[a]

[a] *Institute of Clinical Pharmacology, Goethe-University, Theodor-Stern-Kai 7, D-60590 Frankfurt am Main, Germany*

[b] *Fraunhofer Institute of Molecular Biology and Applied Ecology-Project Group Translational Medicine and Pharmacology (IME-TMP), Theodor-Stern-Kai 7, D-60590 Frankfurt am Main, Germany*

[c] *Smell & Taste Clinic, Department of Otorhinolaryngology, University of Dresden Medical School, Fetscherstr. 74, D-01307 Dresden, Germany*

[d] *Clinic for Gastroenterology, Hepatology and Infectious Diseases, Heinrich Heine University, Moorenstraße 5, D-40225 Düsseldorf, Germany*

Abstract—The amino acid taurine is required for development and functioning of the central and peripheral nervous system where it exerts osmoregulatory, neuromodulatory and anti-apoptotic actions. It is subject to cellular import by the taurine transporter *slc6a6*. Absence of the transporter and consequently, absence of taurine leads to several neurologic deficits and sensory losses. In a *slc6a6* knock-out mouse model, consequences of congenital taurine deficiency were assessed in nociceptive sensory processes. The formalin assay, hot plate assay, and summated generator potentials in response to local nociceptive stimulation with gaseous CO_2 were applied. Reduced responsiveness of *slc6a6*(−/−) mice to nociceptive stimulation was observed in particular to chemical nociceptive stimuli. Scl6a6 knock-out mice spent significantly less time licking the formalin injected paw and displayed smaller amplitudes of the nociceptive nasal mucosa potentials than wild-type mice ($p = 0.002$ and 0.01 respectively). In contrast, withdrawal latencies on a hot plate did not significantly differ, suggesting that intracellular taurine deficits lead in particular to a hyposensitivity of nociceptive sensory neurons sensitive to noxious chemical stimulation. As hereditary absence of taurine affects biological processes of anatomical structure development, the altered nociceptive responses likely reflect consequences of compromised peripheral nervous system development.

Key words: pain, knock-out model, mice.

INTRODUCTION

Taurine is an amino acid involved in cell homeostasis, volume regulation, structural integrity of cell membranes, protein stabilization and stress responses (Huxtable et al., 2000; Lourenco and Camilo, 2002). Humans obtain it from dietary content of animal origin (Stapleton et al., 1997) but can also synthetize it (White and Fishman, 1936; Jacobsen and Smith, 1968), which explains its presence in vegans (Rana and Sanders, 1986). Absence of taurine in species that cannot synthetize it, e.g. cats, leads to neurological abnormalities (Aguirre, 1978; Sturman et al., 1986; Aerts and Van Assche, 2002), due to taurine's role in the development and functioning of the central nervous system (CNS) (Hussy et al., 2000a; Lima et al., 2001; Foos and Wu, 2002; Heller-Stilb et al., 2002; Sergeeva et al., 2003).

While CNS taurine is also synthetized locally in glial cells (Reymond et al., 1996; Pow et al., 2002), astrocytes and neurons (Vitvitsky et al., 2011), its availability in neuronal cells depends on the active transport via high-affinity taurine transporters belonging to the solute carrier family 6 (member 6, *slc6a6* (Uchida et al., 1992; Ramamoorthy et al., 1994)), also contributing to the transport of gamma amino-butyric acid (Tomi et al., 2008). In the CNS, the transporter is found in the spinal cord (Jung et al., 2013) and brain (Sergeeva et al., 2003). Taurine-transporter knock-out mice (*slc6a6*(−/−) (Warskulat et al., 2007b) displayed several neurological symptoms such as a disinhibited striatal network activity (Sergeeva et al., 2007), blindness due to retinal degeneration (Heller-Stilb et al., 2002) and olfactory deficits due to structural abnormalities of the olfactory epithelium (Warskulat et al., 2007a).

The widespread effects of taurine for neuronal processes and sensory functioning imply a possible role in nociception. However, consequences of taurine deficits, especially long-term, for nociception have received less attention. Its role as osmoregulator and neuromodulator in the CNS (French et al., 1986; Saransaari and Oja, 2000) suggests a possible involvement of osmosensitive neurons that express several TRP ion channels including TRPV1 and TRPA1 (Sinke and Deen, 2011), which are also excited by chemical stimuli (Patapoutian et al., 2009). Therefore, the present study assessed the role of taurine in

*Correspondence to: J. Lötsch, Institute of Clinical Pharmacology, Goethe-University, Theodor-Stern-Kai 7, D-60590 Frankfurt am Main, Germany. Tel: +49-69-6301-4589; fax: +49-69-6301-4354.
E-mail address: j.loetsch@em.uni-frankfurt.de (J. Lötsch).

Abbreviations: HSAN, hereditary sensory and autonomic neuropathy; NMP, negative mucosa potential; rm-ANOVA, analysis of variance for repeated measures.

0306-4522/13 $36.00
http://dx.doi.org/10.1016/j.neuroscience.2013.11.037

nociceptive sensory responses to chemical stimuli in a taurine transporter (*slc6a6*) knock-out mouse model.

EXPERIMENTAL PROCEDURES

Animals

The taurine transporter knock-out mice, *slc6a6*(−/−), were generated by homologous recombination. They had a mixed genetic background (C57Bl/6 × 129/SvJ) as described previously (Heller-Stilb et al., 2002). Wild-type mice, *slc6a6*(+/+) served as control animals. Mice were aged approximately 6 months; body mass of wild-type and knock-out mice was 29 ± 4 g (arithmetic mean and standard deviation) and 24 ± 2 g, respectively. Mice were bred and maintained in the animal facility of the University of Düsseldorf, Germany, and shipped to the Goethe University, Frankfurt am Main, Germany, at least 2 weeks before testing. Mice were maintained on a 12/12-h light/dark cycle, had free access to food and water and were kept at controlled temperature before and during the experiments. Experiments were conducted according to the ethical guidelines for research on laboratory animals (Zimmermann, 1983). Investigator-blindness and a randomized order of knock-out and wild-type mice were applied in the experiments.

Behavioral nociceptive assessments of chronic slc6a6 deficiency

The formalin test response (Dubuisson and Dennis, 1977) reflects during its early phase A_δ-fiber activation due to peripheral nociceptive stimulation while the second phase appears to be dependent on the combination of an inflammatory reaction in the peripheral tissue, sustained C-fiber activation and functional changes in the dorsal horn of the spinal cord resulting in sensitization (Tjolsen et al., 1992). Following injection of 20 µl of a 5% formaldehyde solution into the subcutaneous space at the dorsal side of the left hind paw (n = 12; 6 *slc6a6*(−/−) and 6 *slc6a6*(+/+)), the number of paw liftings and the time spent licking the formalin-injected paw was recorded in 1-min intervals up to 45 min starting right after formalin injection. Test results were expressed as sum of the total number of flinches and the licking time, separately for the early (0–5 min) and late (20–45 min) test phases (Dubuisson and Dennis, 1977), normalized for time.

In addition, hot plate tests were used for comparison with chemical stimulation. This test mainly stimulates heat-responsive nociceptive neurons and reportedly quantifies nociception mediated primarily by supraspinal mechanisms (South and Smith, 1998) with licking or jumping responses being considered as results of supraspinal sensory integration (Caggiula et al., 1995; Rubinstein et al., 1996). At two different days, mice (n = 16; 8 *slc6a6*(−/−) and 8 *slc6a6*(+/+)) were placed at a round metal surface heated to temperatures of 52 °C (day 1) and 55 °C (day 2; Hot Plate FMI, Föhr Medical Instruments GmbH, Seeheim/Ober-Beerbach, Germany; time resolution 0.1 s). Two temperatures were used according the laboratory's standard to address possible changes in the perception versus stimulus strength relationship (Fechner, 1860) between genotypes. The time was recorded until the mouse showed the first behavioral sign of nociception such as (i) licking a hind paw, (ii) vocalization, or (iii) an escape response (South and Smith, 1998). The average of three test repetitions (interval 30 min) was submitted to statistical analysis. To control for differences in motor coordination and balance as a consequence of skeletal muscle damage (Warskulat et al., 2004), Rota rod tests were done using a rubber coated horizontal metal rod (3 cm diameter) rotating at a constant speed of 24 rpm (Ugo Basile, Comerio, Italy), using a cut-off of 90 s.

Electro-physiological assessments of chronic slc6a6 deficiency

In addition to the behavioral assessments, *slc6a6* associated changes in nociceptive input at peripheral receptor level were assessed by means of pain-related summated generator potentials recorded from the nasal mucosa (negative mucosa potential, NMP (Kobal, 1985; Thürauf et al., 1991)) in response to short pulses of gaseous carbon dioxide (CO_2, 65% v/v, duration 1 s, stimulus rise time < 20 ms). CO_2 stimuli specifically excite nasal nociceptors and have therefore been proposed as a model of or the quantitative physiological and pharmacological examination of chemically induced nociception (Anton et al., 1991). The NMP is characterized by a correlation with CO_2 concentrations and, in humans, with the evoked pain, and its amplitudes decrease following local administration of anesthetics and systemic administration of analgesics and are therefore interpreted as a summated receptor potential from chemical nociceptors (Kobal, 1985). In the present experiments, the CO_2 stimuli were embedded in a constantly flowing air stream (2 l/min, 36.5 °C, 80% relative humidity) provided by an olfactometer ((Kobal, 1981) OM2s; Burghart Instruments, Wedel, Germany). CO_2 stimuli were delivered via a Teflon tube (inner diameter 2 mm, length 80 mm) positioned in the rostro-caudal direction at an angle about 30° from the horizontal plane, 2 cm from the septal ridge (distance 2 cm). The electrophysiological recordings (NMPs) were obtained in supravital mice (Gudziol et al., 2010) (n = 14; 4 *slc6a6*(−/−), 5 *slc6a6*(−/+), and 5 *slc6a6*(+/+)). After killing by cervical dislocation heads were cut median-sagitally and the mucosa of the lateral nasal walls was prepared and kept moist with saline. The preparations were stored at 4 °C and allowed to warm up to room temperature (of 22 °C) for at least 20 min before the recordings. Responses to the CO_2 stimuli were recorded from the mucosa by means of tubular electrodes made from Teflon tubing (Labokron, Sinnsheim, Germany; outer diameter 0.8 mm), filled with 1% Ringer-agar and containing a silver-chlorided silver wire (resistance < 20 kΩ). An indifferent Ag–AgCl electrode was placed subcutaneously over the occipital bone. Using a manipulator this electrode was placed at three different sites of the nasal mucosa (superior, middle, and inferior turbinates) to get a representative measure of the entire mucosa (randomized sequence of