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PLASTICITY OF SPINAL CORD GLIAL CELL EXPRESSION/FUNCTION AND SPINAL GLUTAMATE TRANSPORTERS IN CATS DIAGNOSED WITH THE CHRONIC BLADDER PAIN SYNDROME INTERSTITIAL CYSTITIS

Hypothesis / aims of study

Recent evidence has shown that glial cells are involved in pain enhancement in various chronic pain conditions. Activation of astrocytes and microglia may result in altered cell morphology, changes in surface membrane and cytoplasmic protein expression and release of factors which can lead to changes in neuronal function. Interstitial cystitis (IC) is a painful disease which affects bladder function in both cats and humans. This study evaluated the morphology and expression patterns of spinal cord glial cells (astrocytes/microglia) from normal cats and cats diagnosed with IC. In addition, because of the importance of glutamate release from afferent fibers in nociception and neurotoxicity, we also evaluated the involvement of GLAST, a glutamate transporter expressed in astrocytes, which via regulation of glutamate uptake/release could play a role in the neuronal abnormalities in IC.

Study design, materials and methods

Healthy, age-matched cats obtained from commercial suppliers were evaluated and determined to be free from disease and signs referable to the lower urinary tract. Both healthy cats and cats diagnosed with interstitial cystitis (according to the NIDDK criteria) were housed in stainless steel cages and allowed to acclimatize to their environment for at least 3 months before study. Sacral (S1, S2) spinal cord segments were dissected from deeply anesthetized (induction with 2% halothane and then maintained with α-chloralose 60–70 mg/kg) cats (*n*=3 of either sex), and after removal of tissue, the animals were sacrificed by an overdose of the anesthestic. The degree of anesthesia was determined to be adequate for surgery by periodically testing for the absence of a withdrawal reflex to a strong pinch of a hind paw and absence of an eye blink reflex to tactile stimulation of the cornea. Tissues were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2) at 4C overnight, and then transferred to 30% sucrose in PBS (2 days at 4C) prior to freezing . Transverse sections (6 µm thickness) were cut on a cryostat microtome and thaw-mounted on charge-slides.

Immunostaining: An indirect immunofluorescence method was used for glial cell visualization and identification. Transverse spinal cord cryosections (6 µm) were incubated with primary antibodies against the macrophage/microglial specific plasma-membrane protein, CD1b/c (OX-42,1:100-1:200;Cederlane Laboratories) or the major intermediate filament protein in the astrocyte cytoskeleton, glial fibrillary acidic protein (GFAP ,1:100-1:200; Sigma). Double labeling with antibodies against GFAP and the intermediate filament protein, nestin (1:100-1:200, Abcam), was used to identify reactive astrocytes. In addition, because of the importance of glutamate release in chronic pain conditions, we also examined the expression pattern of the astrocyte specific plasma membrane glutamate/aspartate transporter, GLAST (1:100-1:200, Abcam). Fluorescent secondary antibodies (FITC; Cy-3) were used to visualize reaction product and nuclei were visualized by incubation with DAPI (1: 5000, Molecular Probes). Non-specific staining was assessed in the absence of primary antibodies.

Photomicroscopy: Images of immunofluorescence labeling were captured with a Leica DC 200 digital camera (Leica; Heerbrugg, Switzerland) attached to a Zeiss Axioplan microscope (Zeiss; Oberkochen, Germany), fitted with the following filters: Cy3, 510–550 nm excitation, 590 nm emission; FITC, 470 nm excitation and 525 nm emission and DAPI, 358nm excitation and 460nm emission . Images were visualized and saved using C.Imaging software (Compix Inc. Imaging Systems, Pennsylvania, USA).

Results

Our findings revealed that in IC cat spinal cord, grey matter astrocytes (labelled with GFAP) had a more stellate appearance and expressed the intermediate filament nestin, a marker for the reactive state. In addition, astrocytes in IC spinal cord exhibited an increased density of GFAP distribution throughout dorsal horn, lamina X surrounding the central canal as well as the autonomic region (lamina V-VII). This finding is consistent with the reported hypertrophy and hyperplasia of reactive astrocytes. Microglial cell (labelled by OX-42) processes showed signs of retraction and were less pronounced when compared with normal cat spinal cord, which is also indicative of a reactive state.

There was no observable difference in expression density of the astrocyte specific glutamate transporter (GLAST) in fibrous astrocytes (white matter) from either IC or normal cat spinal cord. By contrast in IC animals, protoplasmic astrocytes (grey matter) exhibited a higher GLAST expression than in normal spinal cord tissue.

Interpretation of results

Previous reports have demonstrated that IC cat afferent neurons exhibit abnormal responses to chemical and mechanical stimulation. In addition, a number of studies have suggested that changes in spinal cord glial cell expression/function may be an important factor in a number of chronic pain conditions. The present study demonstrated that IC cat spinal cord glial cells (astrocytes/microglia) show alterations in expression patterns as compared to spinal cord glial cells from normal cats. In addition, glial cells from IC cats also express markers for the “reactive” state.

The neurotransmitter glutamate is released from afferent nerves and plays a significant role in pain processing and neurotoxicity. The astrocyte-specific glutamate transporter (GLAST) is thought to be involved in the regulation of glutamate (uptake/release) in CNS interstitium. The present results also demonstrate that IC cat astrocytes exhibit increased expression of GLAST. Taken together, these findings suggest that alterations in spinal cord glia cell expression/function as well as glutamate uptake/release may play a role in the changes in neural function and painful bladder symptoms in IC.

Concluding message

These findings suggest that plasticity in spinal cord glial cell expression/function may be linked with the painful bladder symptoms in interstitial cystitis. Pharmacologic interventions aimed at targeting neuronal-glial interactions may provide a new prospective on clinical management of chronic bladder pain disorders.

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ANIMAL SUBJECTS: This study followed the guidelines for care and use of laboratory animals and was approved by University of Pittsburgh and Ohio State University