Downregulation of spinal astrocytic connexin43 leads to mechanical hypersensitivity through upregulation of interleukin-6 and cyclooxygenase-2 in mice

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Backgrounds

Connexin43 (Cx43) is expressed in spinal dorsal horn astrocytes and crucial in the maintenance of neuropathic pain. Downregulation of spinal astrocytic Cx43 enhances glutamatergic neurotransmission, resulting in cutaneous hypersensitivity in mice. Decreased expression of astrocytic Cx43 could lead to altered expression of other nociceptive molecules. Then, the current study has elaborated mechanisms mediating nociceptive hypersensitivity due to Cx43 downregulation.

Methods

Primary spinal astrocytes were prepared from the spinal cord of neonatal Wistar rats. Knockdown of Cx43 in cultured astrocytes and spinal dorsal horn of ddy mice were performed by using RNA interference. Expression levels of mRNA or protein were measured by real-time PCR or Western blot, respectively. The withdrawal threshold of the hind paw to mechanical stimulation was determined using von Frey filaments.

Results

Transfection of Cx43-targeting siRNA in cultured spinal astrocytes increased expression of the pronociceptive cytokine interleukin-6 (IL-6) and the prostaglandin synthesizing enzyme cyclooxygenase-2 (COX-2). Increased expression of IL-6 and COX-2 was due to decreased Cx43 expression rather than due to diminished Cx43 channel function. In mice, downregulation of spinal Cx43 expression by intrathecal treatment with Cx43-targeting siRNA increased IL-6 and COX-2 expression and induced hind paw mechanical hypersensitivity. Connexin43 siRNA-induced mechanical hypersensitivity was attenuated by intrathecal treatment with anti-IL-6 neutralizing antibody and intraperitoneal treatment of selective COX-2 inhibitor celecoxib. Suppression of glycogen synthase kinase-3 β (GSK-3 β), a serine/threonine protein kinase, prevented upregulation of IL-6 and COX-2 expression induced by Cx43 downregulation in both cultured astrocytes and in mouse spinal dorsal horn. Inhibition of spinal GSK-3 β also ameliorated Cx43 siRNA-induced mechanical hypersensitivity.

Conclusions

The current findings indicate that downregulation of spinal astrocytic Cx43 leads to changes in expression of IL-6 and COX-2 underlying the maintenance of pain following peripheral nerve injury.