EFFECT OF ADENOSINE ON HYPERALGESIA AFTER SPINAL CORD INJURY

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Introduction
After spinal cord injury, severe sensory disturbances occur, accompanied by motor deficiencies. Some patients with spinal cord injury complain of feeling several kinds of pain sensations. Allodynia, hyperalgesia, and continuous spontaneous pain are typical chief complaints. Along with other type of neuropathic pain, spinal cord damage-induced pain is usually unresponsive to conventional treatments. The pain sensations have been believed to be controlled by the balance between excitatory and inhibitory signalings. The typical stimulatory signalings are glutamate, substance P, and inhibitory signalings are serotonin, noradrenalin and GABA. Adenosine, an endogenous neuromodulator, controls neuronal membrane potential via adenosine receptors. One of the actions of adenosine receptors is hyperpolarization of the cell membrane that inhibits the excitation of neurons. The purpose of this study is testing adenosine on hyperalgesia after the spinal cord injury.

Materials and Methods
Spinal cord mild-compression model (SCI model) and drug application
Under general anesthesia by halothane, the rat spinal cord was carefully exposed by removing the vertebral lamina at the 11th vertebrae. Direct compression was performed using a 20 g weight of which the point of contact to the dura consisted of very soft and rounded silicone. The weight was gently placed on the thoracic spinal cord extradurally for 20 minutes (SCI). We did not observe any serious damage such as hyperextension, paresis of the hind limbs, or histological hemorrhage with tissue destruction at the point of compression. In some experiments, a laminectomy of the 11th vertebrae was performed without spinal cord compression (sham). 10 µl of the drug was injected into subarachnoid space at the 4-5th lumbar intervertebral level one hour before the measurement of heat hyperalgesia.

Evaluation of thermal hyperalgesia
To evaluate the withdrawal threshold to paw thermal stimulation, we used the Hargreaves’s plantar test apparatus (Ugo Basile, Varese, Italy). Rats were placed on a 2 mm thick glass floor, a mobile infrared heat generator with an aperture of 10 mm in diameter was aimed at the rat’s hind paw under the floor. When the rats felt pain and withdrew their paw, the power shut off and the reaction time (the withdrawal latency of the paw) was recorded automatically. Shortening of the withdrawal latency indicated thermal hyperalgesia. The temperature of the glass floor was kept at 22.5-23.5°C.

Data analysis
For statistical analysis of the data, an analysis of variance (ANOVA) followed by Fisher’s PLSD was used.

Results
In the normal rat without operation, hyperalgesia was induced by the intrathecal application of 10 mg DPCPX, a selective antagonist of adenosine A1 receptor. On the other hand, intrathecal application of 30 nmol ZM241385, a selective antagonist of adenosine A2a receptor, did not change the withdrawal latency. These results suggest that continuous stimulation of A1 receptor by endogenous adenosine maintains normal sensory condition. Three days after the injury, significant heat-hyperalgesia was observed in the hindlimbs paws of SCI rats. Hyperalgesia, induced by spinal cord injury, was significantly inhibited by the intrathecal application of 10 to 30 nmol Cl-adenosine, an unselective adenosine receptor agonist (Fig. 1). The effect of Cl-adenosine (10 nmol) on the hyperalgesia after the spinal cord injury was blocked by the simultaneous application of DPCPX (Fig. 2). Intrathecal application of R-PIA (10 nmol), an agonist of A1 receptor, also inhibited spinal cord injury-induced hyperalgesia. On the other hand, intrathecal application of CGS21680, a selective agonist of adenosine A2a receptor, did not inhibit the spinal cord injury-induced hyperalgesia.

Discussion
In thoracic spinal cord injury, dysfunctions occur because of damage to descending and ascending pathways. Serotonin and noradrenalin are typical inhibitory transmitters in the descending pathways. We have reported that inhibition of serotonergic descending pathways induces hyperalgesia after the spinal cord injury [1]. Adenosine acts as cell modulator influencing via several receptor-mediated effects. A1 and A2 receptors are found in the central nervous system associated with neurons as well as with glial cells. High affinity A1 and A2a receptors are activated in nanomolar concentrations of adenosine. The A2 receptors are coupled to the adenylcyclase, stimulating cAMP elevation that is inhibited by A1 receptors. The functionally important regulation of membrane ion channels seems to be the domain of the A1 receptors that activate specific hyperpolarizing K+ and Cl conductance. This counteracts, via synaptic and extrasynaptic receptors, nerve cell depolarisation [2]. The idea of the present study was that the hyperpolarizing effect of adenosine could inhibit excitation of sensory nerve. We showed that the blockage of the adenosine A1 receptor by intrathecal injection of selective A1 receptor antagonist (DPCPX) mimicked thermal-hyperalgesia. This fact indicates that the adenosine signal suppresses activity of the pain pathway under normal physiological conditions.

In the SCI rats, intrathecal application of Cl-adenosine inhibited the hyperalgesia in a dose-dependent manner. This action of Cl-adenosine was blocked by the simultaneous application of A1 receptor antagonist (DPCPX). This result suggests that the anti-hyperalgesic action of Cl-adenosine was mediated by the stimulation of A1 receptor. We confirmed the A1-receptor-mediated anti-hyperalgesic action using selective A1 receptor agonist (R-PIA). The intrathecal application of R-PIA significantly inhibited the hyperalgesia after the spinal cord injury. On the other hand, no effect was seen using A2a receptor agonist. The hyperpolarizing effect of A1 receptor may counteract the neuronal excitation caused by spinal cord injury.

In this study, we have demonstrated the anti-pain effect of adenosine A1 receptor stimulation. For clinical use, the enhancement of adenosine signals by, i.e, the application of A1 receptor agonists, adenosine reuptake inhibitors, adenosine kinase inhibitors and adenosine deaminase inhibitors may become a candidate for the development of novel therapeutic methods against neuropathic pain after spinal cord injury.

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