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Intrathecal administration of a gap junction decoupler, an inhibitor of Na^+–K^+–$2Cl^-$ cotransporter 1, or a $GABA_A$ receptor agonist attenuates mechanical pain hypersensitivity induced by REM sleep deprivation in the rat

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ABSTRACT

We studied the hypothesis that some of the spinal mechanisms that are involved in neuropathic hypersensitivity play a role in hypersensitivity induced by REM sleep deprivation (REMSD). Rats with a chronic intrathecal (i.t.) catheter had REMSD of 48 h duration that induced hypersensitivity to mechanical stimulation. After REMSD, the animals were treated i.t. with carbenoxolone (a gap junction decoupler), bumetanide (a blocker of Na^+–K^+–$2Cl^-$ cotransporter 1 or NKCC1), muscimol (a $GABA_A$ receptor agonist), or pretreated intraperitoneally with minocycline (an inhibitor of microglia activation). Previously, all these treatments attenuated neuropathic hypersensitivity. Following REMSD, carbenoxolone, bumetanide and muscimol had a strong antihypersensitivity effect, whereas pretreatment with minocycline failed to prevent development of hypersensitivity. The results suggest that among spinal pain facilitatory mechanisms that are common to REMSD and neuropathy are NKCC1 blocker- and gap junction decoupler-reversible mechanisms. Moreover, there is a net pain inhibitory effect by spinal administration of an exogenous $GABA_A$ receptor agonist following REMSD as shown earlier in neuropathy. In contrast, activation of spinal microglia may not be as important for the development of hypersensitivity induced by REMSD as following nerve injury.

1. Introduction

There is considerable amount of evidence indicating that sleep deprivation can induce pain and hyperalgesia both in clinical and experimental conditions (Lautenbacher et al., 2006). In experimental animals, sleep deprivation is frequently induced by the flower pot technique that leads to deprivation of rapid eye movement (REM) sleep (Morden et al., 1967) and pain hypersensitivity (e.g., Damasceno et al., 2009; Hicks et al., 1978; Onen et al., 2000; Wei et al., 2008). Spinal mechanisms may contribute to pain hypersensitivity induced by REM sleep deprivation (REMSD). Moreover, some of the spinal mechanisms that underlie REMSD-induced pain hypersensitivity may be, at least partly, the same that contribute to pain hypersensitivity in nerve-injured animals. This is indicated by finding that REMSD is followed by facilitation of spinal withdrawal responses elicited by noxious stimulation. Moreover, pain hypersensitivity induced by REMSD has been reduced by intrathecal (i.t.) administration of a glutamatergic receptor antagonist or a nitric oxide synthase inhibitor at a dose that failed to influence pain behavior in healthy controls (Wei et al., 2007). Potential neural substrates for mediating the influence of REMSD to the spinal pain circuitry are the brainstem structures involved in control of both sleep (McCarley, 2007) and pain (Pertovaara and Almeida, 2006) and that have efferent projections to the spinal cord; among such brainstem structures are, for example, the noradrenergic locus coeruleus and the serotoninergic raphe nuclei.

Among spinal mechanisms contributing to injury-induced pain hypersensitivity is neuroinflammation, in which microglia and release of cytokines or other inflammatory mediators play a significant role (Hansson, 2010; McMahon et al., 2006). Pronociceptive molecules released by activated microglia include growth factors, such as brain-derived neurotrophic factor (BDNF). In addition to protection of neurons (Suter et al., 2007), BDNF, through action on the spinal TrkB receptor, is known to promote pain hypersensitivity (Wang et al., 2009). Moreover, activation of glial cells, particularly astrocytes, has been associated with their coupling to adjacent astrocytes or neurons that may promote spread of excitation (Alvarez-Maubecin et al., 2000). It is not yet known whether activation of spinal microglia or coupling of spinal astrocytes contributes to pain hypersensitivity induced by REMSD.

Transmembrane gradient for chloride ions influences the reversal potential for chloride. The reversal potential for chloride determines whether opening of chloride channels, e.g. by GABA acting on the $GABA_A$ receptor, induces hyper- or depolarization of the neuron (De Koninck,

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2007; Price et al., 2009). When intracellular chloride concentration is high as it normally is in primary somatosensory neurons, GABA induces depolarization of their central terminals that is considered to contribute to presynaptic inhibition of the sensory signal (Rudomin and Schmidt, 1999; Willis, 1999). When intracellular chloride concentration is low as it normally is in sensory interneurons, GABA induces hyperpolarization of the sensory interneuron. Inwardly directed Na^+–K^+–Cl^- cotransporter 1 (NKCC1) contributes to high intracellular Cl^- concentration in primary sensory neurons, whereas outwardly directed K^+–Cl^- cotransporter 2 (KCC2) contributes to low intracellular Cl^- concentration in interneurons (Russell, 2000). Earlier studies have shown that increase in BDNF (Rivera et al., 2002) and peripheral nerve injury or inflammation (Coull et al., 2003; Cramer et al., 2008; Miletic and Miletic, 2008; Zhang et al., 2008) are among factors that induce down-regulation of KCC2 in the spinal dorsal horn. After down-regulation of KCC2, GABA acting on the $GABA_A$ receptor may produce excitatory rather than inhibitory action on the pain-relay neuron (Coull et al., 2003). On the other hand, nerve injury or inflammation has increased phosphorylation, membrane mobilization and expression of NKCC1 in the spinal cord (Cramer et al., 2008; Galan and Cervero, 2005). It has been proposed that the net effect following increased activity of NKCC1 in central terminals of primary afferent nerve fibers is their excessive depolarization and generation of action potentials in pain pathways rather than presynaptic inhibition of the sensory signal; this type of mechanism presumably contributes to activation of pain pathways by touch (Cervero and Laird, 1996). In line with this proposal, a blocker of NKCC1, bumetanide, has attenuated inflammatory and neuropathic hypersensitivity (Cramer et al., 2008; Granados-Soto et al., 2005; Pitcher et al., 2007; Valencia-de Ita et al., 2006). It still remains to be studied whether a blocker of NKCC1 has an antihypersensitivity effect also following REMSD.

In the present study, we attempted to determine whether coupling of glial cells, activation of microglia, NKCC1 or a change in the GABAergic regulation of the chloride channel in the spinal cord plays a role in REMSD-induced pain hypersensitivity. For this purpose, pain behavior was assessed in REM sleep-deprived animals and healthy controls that were treated with compounds that influence glial cell coupling, activation of microglia, NKCC1 or the $GABA_A$ receptor.

2. Materials and methods

2.1. Experimental animals

The experiments were performed in adult, male Hannover–Wistar (HW) rats (weight: 150–200 g; CAS, Shanghai, China). All experiments were approved by the institutional ethics committee and all experimental procedures are in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985). All efforts were made to minimize animal suffering and to use only the number of animals necessary to produce reliable scientific data.

2.2. Techniques for microinjection

For intrathecal (i.t.) drug injections a catheter (PE-10) was administered into the lumbar level of the spinal cord under pentobarbital anesthesia (50 mg/kg i.p.) as described in detail elsewhere Størkson et al., 1996). Following recovery from anesthesia, the correct placing of the catheter was verified by administering lidocaine (4%, 7–10 μl followed by a 15 μl of saline for flushing) with a 50 μl Hamilton syringe. Only those rats that had no motor impairment before lidocaine injection but had a bilateral paralysis of hind limbs following i.t. administration of lidocaine were studied further. For i.t. administration, the drugs were microinjected with a 50 μl Hamilton microsyringe in a volume of 5 μl followed by a saline flush in a volume of 15 μl.

2.3. REM sleep deprivation procedure

The pedestal-over-water or flower pot technique of REM sleep deprivation was modified from the method described earlier (Morden et al., 1967). Briefly, rat was placed on top of platform surrounded by water. The base of the cage was submerged in 4 cm of water. The platform was 7.5 cm in diameter and 7.5 cm high. REM sleep deprivation was performed for 48 h. The rat was allowed to recover from sleep deprivation for at least one week before next testing.

Under control conditions, the animals were living in similar cages (one animal/cage) as during sleep deprivation, except that there was no flower pot or water in the cage.

2.4. Behavioral testing

To assess mechanical hypersensitivity, the frequency of the withdrawal response to the application of monofilaments (von Frey hairs) to the hind paw was examined. Nine hairs with forces of 1–60 g (Stoelting, Wood Dale, IL) were applied five times at a frequency of approximately of 0.5 Hz. Hairs were tested in ascending order of force. A visible lifting of the stimulated hind limb was considered a withdrawal response. The focus was on mechanical sensitivity, since our earlier study indicated that REM sleep deprivation has a more pronounced effect on mechanical than heat sensitivity (Wei et al., 2007). Moreover, central mechanisms that were studied in our experiments play an important role in hypersensitivity to mechanical stimulation (Treede et al., 1992).

2.5. Motor performance test

To exclude the possibility that the drug-induced effects on pain behavior were due to motor rather than sensory action, the potential motor impairment by the studied compounds was assessed in a Rotarod test. In the test, the animals were placed on a revolving drum (a constant speed of 26 rounds/min) of a Rotarod device (Ugo Basile, Varese, Italy). The latency until the animal dropped from the drum was determined with a stop watch. Before any drug testing, the rats were habituated to the Rotarod test during two previous days. The maximum observation period was 1 min after which the animal that was still on the drum was removed. The Rotarod test was repeated three times at 1 min intervals and the longest latency for each rat in each condition was used in further calculations.

2.6. Drugs

Carbenoxolone (a gap junction decoupler), bumetanide (a blocker of Na^+–K^+–$2Cl^-$ cotransporter 1), muscimol (a $GABA_A$ receptor agonist), and minocycline (an inhibitor of microglia activation) were purchased from Sigma-Aldrich (St. Louis, MO). Drugs were dissolved in saline. Physiological saline was used as control. Minocycline was administered intraperitoneally, while other compounds were administered intrathecally.

2.7. Course of the study

In a preliminary test, in which the hypersensitivity effect induced by REMSD *per se* was assessed, pain behavior was assessed 24 h and 48 h following REM sleep deprivation. Drug effects on pain-related behavior were assessed in two experimental conditions: 1) 48 h after REM sleep deprivation (testing started immediately after the end of the sleep deprivation), 2) control conditions without REM sleep deprivation. The monofilament test was performed prior to 5, 15, 30 and 60 min after intrathecal administration of each drug dose or vehicle control, except when testing minocycline (see further discussion). While the experiments were not formally blinded, it should be pointed out that the experimenter assessing pain behavior