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Research report

The role of the dopamine D2 receptor in descending control of pain induced by motor cortex stimulation in the neuropathic rat

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ABSTRACT

We studied in rats with a spinal nerve ligation-induced neuropathy whether dopamine D2 receptors (D2Rs) play a role in descending control of pain induced by stimulation of the primary motor cortex (M1). Noxious heat-evoked responses were determined in spinal dorsal horn wide-dynamic range (WDR) and nociceptive-specific (NS) neurons, with and without electrical M1 stimulation. A D2R antagonist, raclopride, was administered into the dorsal striatum or spinally in attempts to reverse spinal antinociception induced by M1 stimulation. Moreover, influence of M1 stimulation on the noxious heat-induced limb withdrawal reflex was determined following block of spinal D2Rs with raclopride or a lidocaine-induced block of the hypothalamic A11 cell group, the main source of spinal dopamine. Striatal administration of raclopride enhanced the heat-evoked baseline responses of WDR but not NS neurons and reversed the M1 stimulation-induced suppression of the heat response in WDR neurons. Following spinal administration of raclopride, M1 stimulation failed to suppress the heat response of WDR neurons, whereas the heat response of NS neurons was enhanced by M1-stimulation. After blocking the A11 with lidocaine or spinal D2Rs with raclopride, M1 stimulation failed to suppress the noxious heat-evoked withdrawal reflex. The results indicate that descending pain control induced by stimulation of the M1 cortex in neuropathic animals involves supraspinal (presumably striatal) and, through A11, spinal D2Rs. Supraspinal and spinal D2Rs have partly dissociative effects on spinal dorsal horn WDR and NS neurons, possibly reflecting differential roles and wirings that these sensory neurons have in pain-processing circuitries.

1. Introduction

Electrical stimulation of the primary motor cortex (M1) has suppressed pain-related responses in neuropathic (Fonoff et al., 2009; Lucas et al., 2011; Pagano et al., 2011; Vaculin et al., 2008; Viisanen and Pertovaara, 2010a,b) as well as healthy control animals (Rojas-Piloni et al., 2010; Senapati et al., 2005; Viisanen and Pertovaara, 2010a). Transcranial magnetic stimulation of M1 attenuated pain perception also in healthy human subjects (Johnson et al., 2006; Nahmias et al., 2009). In the clinic, invasive electrical or non-invasive transcranial magnetic stimulation of M1 has been used for alleviation of intractable chronic pain in human patients (Fontaine et al., 2009; Hosomi et al., 2008; Lazorthes et al., 2007; Lefaucheur, 2008; Nguyen et al., 1999; Tsubokawa et al., 1991) with reported success rates of treatment varying from about 40% to 80% (Garcia-Larrea and Peyron, 2007; Lima and Fregni, 2008; Nuti et al., 2005).

While supraspinal mechanisms influencing perception or the emotional appraisal of pain may have an important role in the M1 stimulation-induced pain relief particularly in human studies (Garcia-Larrea and Peyron, 2007; Maarrawi et al., 2007; Peyron et al., 2007), spinal antinociceptive effects in experimental animals (Pagano et al., 2011; Senapati et al., 2005; Viisanen and Pertovaara, 2010a) indicate that brainstem-spinal pathways may also play a role in the M1 stimulation-induced suppression of pain behavior. In line with this, spinal antinociception induced by M1 stimulation in experimental animals was attenuated by blocking the opioid (Fonoff et al., 2009) or the 5-HT_{1A} receptor (Viisanen and Pertovaara, 2010b), both of which are known to play a role in descending pain regulation (Pagano et al., 2011). Moreover, the M1 stimulation-induced spinal antinociception was associated with increased activation of neurons in a number of brainstem structures that are involved in pain control, such as the periaqueductal gray (Pagano et al., 2011). However, the noradrenergic pontospinal pathways are not among them as indicated by the failure to reduce the M1 stimulation-induced spinal antinociception with an α_2-adrenoceptor antagonist (Viisanen and Pertovaara, 2010a). A recent study suggested that corticospinal pathways may also directly mediate the M1 stimulation-induced spinal antinociception (Rojas-Piloni et al., 2010) and another study showed that the zona incerta nucleus in the thalamus was involved in the M1

Abbreviations: A11, hypothalamic A11 cell group; D2R, dopamine D2 receptor; M1, primary motor cortex; NS, nociceptive-specific; WDR, wide-dynamic range.

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stimulation-induced spinal antinociception in animals with an experimental model of central pain (Lucas et al., 2011).

Dopamine acting on the dopamine D2 receptor (D2R) has a role in pain control at various central sites (Wood, 2008). One of them is the dorsal striatum, where a D2R agonist reduced spinal nociception (Ansah et al., 2007; Lin et al., 1981; Magnusson and Fisher, 2000; Saunier-Rebori and Pazo, 2006), whereas lesions of the striatal dopaminergic system produced pain hypersensitivity (Chudler and Lu, 2008; Saadé et al., 1997; Takeda et al., 2005). Since the M1 has efferent connections to the striatum (McGeorge and Faull, 1989) and M1 stimulation induces striatal release of dopamine (Kanno et al., 2004; Nieoullon et al., 1978; Strafella et al., 2003), it may be speculated that dopamine acting on the striatal D2R is involved in mediating the M1 stimulation-induced spinal antinociception. In the hypothalamus, the dopaminergic A11 cell group projects to the spinal cord (Hökfelt et al., 1979; Qu et al., 2006; Skagerberg and Lindvall, 1985) where it produces antinociception, due to action on the spinal D2R (Fleetwood-Walker et al., 1988; Taniguchi et al., 2011; Wei et al., 2009). It is not yet known whether A11 or the spinal D2R contribute to the M1 stimulation-induced spinal antinociception.

Here we studied whether dopamine acting on the D2R contributes to the spinal antinociception induced by stimulation of the M1. We addressed the specific hypothesis that the D2R is involved in relaying the descending pain suppressive effect induced by electrical stimulation of the M1 in peripheral neuropathy. For this purpose, we determined whether blocking the D2Rs by dorsal striatal or spinal administration of a D2R antagonist prevents the M1 stimulation-induced spinal antinociception in nerve-injured animals. Furthermore, we also assessed whether blocking the dopaminergic A11 cell group reduces spinal antinociception induced by M1 stimulation.

2. Materials and methods

2.1. Experimental animals

The experiments were performed in adult, male Hannover–Wistar rats (Harlan, Horst, Netherlands; weight: 200–300 g). The Experimental Animal Ethics Committee of the Provincial Government of Southern Finland (Hämeenlinna, Finland) approved methods, and the experiments were performed according to the guidelines of European Communities Council Directive of 24 November 1986 (86/609/EEC). All efforts were made to limit distress and to use only the number of animals necessary to produce reliable scientific data. Rats were housed in a 12-h light/dark cycle with free access to food and water.

2.2. Techniques for producing neuropathy

There are a number of commonly used experimental models of peripheral neuropathy (Honoré et al., 2011), of which we chose for this study that induced by spinal nerve ligation (Kim and Chung, 1992). The unilateral ligation of two spinal nerves (L5 and L6) was performed under sodium pentobarbital anesthesia (50 mg/kg i.p.) as described in detail earlier (Kim and Chung, 1992). Briefly, left paraspinal muscles were separated from the spinous processes at the L4–S2 levels. The L6 transverse process was partly removed to identify visually the L4 and L6 spinal nerves. The left L5 and L6 spinal nerves were isolated and tightly ligated with 6–0 silk thread. After nerve ligation, the wound was sutured and the rats were allowed to recover. Development of nerve injury-induced mechanical hypersensitivity was assessed in unanesthetized animals 10–14 days following the operation. Only animals developing a marked hypersensitivity to mechanical stimulation with monofilaments (hind limb withdrawal thresholds in the operated side < 2 g, which is below the 95% confidence limits of the threshold in healthy controls) and with no motor impairment were selected for the neuropathic study group. Influence of cortical stimulation on neuronal or limb withdrawal responses was studied two to four weeks after ligation of spinal nerves.

2.3. Preparation for electrical stimulation of the primary motor cortex (M1)

For electrical stimulation of M1, a small hole was drilled in the skull for a concentric bipolar electrode (Rhodes NE-100, David Kopf Instruments, Tujunga, CA, USA). The desired stimulation site in M1 was between 1.7 mm anterior and 0.30 mm posterior from the bregma, 1–3 mm lateral from the midline and 0.7–2.2 mm ventrally from the dura mater (Fig. 1A) (Paxinos and Watson, 1986). Electrical stimulations of M1 were performed ipsilateral to the spinal nerve ligation. Although the contralateral M1 area representing the injured region is typically stimulated to produce long-term analgesia in clinical studies (Brown and Barbaro, 2003; Nguyen et al., 1999; Tsubokawa et al., 1991), the M1 cortex ipsilateral to the injured limb was chosen as the stimulation site, since it was considered to reduce potential movement-related artifacts and since also stimulation of the ipsilateral M1 as well as that of the contralateral M1 has produced a significant suppression of pain-related responses in previous human (Nahmias et al., 2009; Poreisz et al., 2008) and animal (Lucas et al., 2011; Senapati et al., 2005; Viisanen and Pertovaara, 2010a,b) studies. Moreover, the focus of the study was on subcortical relays contributing to the cortical regulation of pain rather than on corticospinal pathways that act predominantly on the contralateral side (Canedo, 1997). Chemical or electrical stimulations of the potential subcortical dopaminergic relays studied in this investigation (the dorsal striatum receiving inputs from the ipsilateral M1 (McGeorge and Faull, 1989) and the hypothalamic A11 cell group) have proved to produce significant antinociceptive effects in the ipsilateral side (Ansah et al., 2007; Belforte and Pazo, 2005; Fleetwood-Walker et al., 1988; Pertovaara and Wei, 2008; Saunier-Rebori and Pazo, 2006; Taniguchi et al., 2011; Wei et al., 2009). The differences in stimulus conditions (ipsilateral M1 stimulation in the rat versus predominantly contralateral M1 stimulation in the human patients) and differences in readouts (spinal nociception in the rat versus verbal pain report in the human patient), however, provide limitations when interpreting spinal antinociceptive effects of the present study in terms of pain suppression in clinical patients. While drug effects were not studied on spinal antinociception induced by stimulation of the contralateral M1, one additional control experiment was performed to verify that M1 stimulation, independent of the hemisphere of cortical stimulation (ipsi- versus contralateral to the nerve injury and the spinal dorsal horn neuron), does have a spinal antinociceptive action in neuropathic animals.

Electrical stimuli were generated by a constant current stimulator (PSIU6 and Grass S88, Grass Instruments, Quincy, MA, USA). Electrical stimulation of M1 was performed at the frequency of 300 Hz (duration of each stimulus pulse: 0.1 ms) as in earlier studies (Senapati et al., 2005; Viisanen and Pertovaara, 2010a,b). Since the results of our preliminary experiments indicated that electrical M1 stimulation produced its strongest antinociceptive effect at the intensity of 30 μA, this study focused on assessing the magnitude of spinal antinociception induced by electrical M1 stimulation at the intensity of 30 μA. In a control condition, intensity of M1 stimulation was 0 μA (sham stimulation of M1). Electrical stimulation of M1 started 5 s before heat stimulation of the hind paw and it continued throughout the heat stimulation of 10 s duration. In each experimental condition, the results obtained with and without M1 stimulation in the drug-treatment condition were compared with the results obtained in the identical saline control condition.

2.4. Preparation for cerebral drug injections

For drug delivery into the dorsal striatum or A11, a hole was drilled for a 26-gauge guide cannula (C315G, PlasticsOne, Roanoke, VA, USA). The desired injection site in the striatum was between 0.7 anterior and 0.3 mm posterior from the bregma, 3.4–4.2 mm lateral from the midline and 4.0–6.8 mm from the dura mater (Fig. 1). It should be noted here that although the dopamine in the ventral striatum (nucleus accumbens) is also known to modulate pain behavior (Altier and Stewart, 1999), the striatal target of the present study was the dorsolateral part of the caudate–putamen complex. In the hypothalamic A11, the desired injection site was 3.0 mm posterior from the bregma, 0.6 mm lateral from the midline and 7.5–8.0 mm from the dura mater (Fig. 1B; Paxinos and Watson, 1986). The tip of the guide cannula was positioned 1 mm above the desired injection site in the striatum or A11. Because of proximity of the cortical stimulation site in the M1, the striatal guide cannula was inserted at an angle anterior to the desired injection site in the caudate–putamen complex. Different animals were used when studying effects induced by striatal and hypothalamic injections.

Drugs or saline were microinjected into the striatum or A11 through a 33-gauge stainless steel injection cannula (C315I, PlasticsOne) inserted through and protruding to 1 mm above the tip of the 26-gauge guide cannula (C315G, PlasticsOne). The microinjections were performed using a 10-μl Hamilton syringe (Hamilton Company, Bonaduz, Switzerland) connected to the injection cannula by polyethylene (Intramedic PE-10, Becton Dickinson and Company, Sparks, MD, USA) tubing. The volume of the injections was 0.5 μl. The efficacy of the injection was monitored by watching the movement of a small air bubble through the tubing. The injection lasted 30 s and the injection cannula was left in place at least for an additional 30 s and in most cases, until the next injection was performed.

2.5. Electrophysiological recordings of spinal dorsal horn neurons

Electrophysiological recordings of spinal dorsal horn neurons were performed 2–4 weeks after nerve injury under sodium pentobarbital anesthesia. Anesthesia was induced by administering 50 mg/kg of sodium pentobarbital i.p. and it was continued by administering sodium pentobarbital at the dose of 15–20 mg/kg/h. The level of anesthesia was frequently monitored by assessing the size of the pupils, general muscle tone and by assessing withdrawal responses to noxious stimulation. Supplemental doses of sodium pentobarbital were given as required. The rats were