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Opioid regulation of spinal cord plasticity: Evidence the kappa-2 opioid receptor agonist GR89696 inhibits learning within the rat spinal cord

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Abstract

Spinal cord neurons can support a simple form of instrumental learning. In this paradigm, rats completely transected at the second thoracic vertebra learn to minimize shock exposure by maintaining a hindlimb in a flexed position. Prior exposure to uncontrollable shock (shock independent of leg position) disrupts this learning. This learning deficit lasts for at least 24 h and depends on the NMDA receptor. Intrathecal application of an opioid antagonist blocks the expression, but not the induction, of the learning deficit. A comparison of selective opioid antagonists implicated the kappa-opioid receptor. The present experiments further explore how opioids affect spinal instrumental learning using selective opioid agonists. Male Sprague–Dawley rats were given an intrathecal injection (30 nmol) of a kappa-1 (U69593), a kappa-2 (GR89696), a mu (DAMGO), or a delta opioid receptor agonist (DPDPE) 10 min prior to instrumental testing. Only the kappa-2 opioid receptor agonist GR89696 inhibited acquisition (Experiment 1). GR89696 inhibited learning in a dose-dependent fashion (Experiment 2), but had no effect on instrumental performance in previously trained subjects (Experiment 3). Pretreatment with an opioid antagonist (naltrexone) blocked the GR89696-induced learning deficit (Experiment 4). Administration of GR89696 did not produce a lasting impairment (Experiment 5) and a moderate dose of GR89696 (6 nmol) reduced the adverse consequences of uncontrollable nociceptive stimulation (Experiment 6). The results suggest that a kappa-2 opioid agonist inhibits neural modifications within the spinal cord.

Keywords: GR89696; Opioids; Plasticity; Instrumental learning; Spinal cord

1. Introduction

Recovery after a spinal cord injury is influenced by events that impact neural activity below the injury (Edgerton, Tillakartne, Bigbee, de Leon, & Roy, 2004; Hodgson, Roy, de Leon, Dobkin, & Edgerton, 1994; Wernig, Muller, Nanassy, & Cagol, 1995). Evidence suggests that regular, response-contingent, training can foster recovery (Edgerton et al., 2004; Hook & Grau, 2007), whereas exposure to uncontrollable stimulations has an adverse effect that impedes recovery (Grau et al., 2004). To examine how controllable versus uncontrollable stimulation affects spinal circuits (for a recent review, see Grau et al., 2006), we isolate the lower (lumbo-sacral) spinal cord from the brain by means of a mid-thoracic transection in the rat. When transected (spinalized) rats are given shock to the tibialis anterior muscle of one hind leg whenever the leg is extended (controllable shock), they exhibit a progressive increase in flexion duration that minimizes net shock exposure (Grau, Barstow, & Joynes, 1998). Evidence indicates that this learning depends on the relationship between leg position (the response) and shock onset (the reinforcer) and reflects a simple form of instrumental conditioning. Interestingly, when shock is applied independent of leg position (uncontrollable shock), rats not only fail to learn, they exhibit a learning deficit that blocks subsequent instrumental

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learning for up to 48 h (Crown, Joynes, Ferguson, & Grau, 2002; Grau et al., 1998).

Further work has verified that instrumental learning depends on neurons within the lumbo-sacral (L4-S2) spinal cord and involves a form of NMDA receptor (NMDAR) mediated plasticity (Joynes, Janjua, & Grau, 2004; Liu, Crown, Miranda, & Grau, 2005). Prior exposure to uncontrollable stimulation appears to inhibit this learning through an opioid-mediated process. Supporting this, we have shown that pretreatment with the opioid antagonist naltrexone blocks the deficit in a dose-dependent fashion (Joynes & Grau, 2004). When exposure to uncontrollable stimulation and instrumental testing were separated by 24 h, we found that administration of naltrexone prior to testing restored the capacity for learning. However, naltrexone had no effect when it was given a day earlier, prior to the period of uncontrollable stimulation. Thus, the opioid antagonist blocked the *expression* of the deficit, but not its *induction*. Recognizing that naltrexone could act at the mu, delta, or kappa-opioid receptor, we compared the relative impact of selective opioid antagonists. Only a kappa antagonist (nor-BNI) blocked the deficit. This pattern of results suggests that exposure to uncontrollable shock produces a lasting modification (a form of memory) that inhibits learning through a kappa-opioid-mediated process. This fits well with our earlier observation that instrumental learning depends on a form of NMDAR-mediated plas ticity (Joynes et al., 2004), because other physiological effects that depend on this form of plasticity (e.g., long-term potentiation [LTP]) are inhibited by kappa-opioids (Caudle, Chavkin, & Dubner, 1994; Caudle, Mannes, & Iadarola, 1997; Terman, Drake, Simmons, Milner, & Chavkin, 2000; Terman, Wagner, & Chavkin, 1994).

Because opioids can inhibit pain (nociceptive) signals within the dorsal horn of the spinal cord, we posited that the learning deficit might be the result of nociceptive inhibition (antinociception). Contrary to this hypothesis, we found that intermittent shock treatments that induce a learning deficit do not produce antinociception (Crown et al., 2002). Moreover, exposure to a different shock regimen (a long, continuous, shock) that produces a robust antinociception, as measured by tail-withdrawal from radiant heat (the tail-flick test), does not inhibit instrumental learning. Further analysis revealed that, rather than inhibiting nociceptive reactivity, exposure to intermittent/uncontrollable stimulation has a sensitizing effect that enhanced responsiveness to mechanical stimulation (Ferguson, Crown, & Grau, 2006). This phenomenon, known as *allodynia*, is regularly observed in response to peripheral inflammation and has been linked to the development of neuropathic pain (Coderre, 1993; Dickenson, 1996; Willis, 2001; Willis, Sluka, Rees, & Westlund, 1996).

Inflammatory agents (e.g., administration of capsaicin into one hind paw) are thought to enhance mechanical reactivity through an NMDAR-dependent increase in neural excitability within the spinal cord, a phenomenon known as *central sensitization* (Ji, Kohno, Moore, & Woolf, 2003; Willis, 2001; Willis et al., 1996). We hypothesized that uncontrollable shock might disrupt learning because it induces a similar state. Supporting this, we showed that peripheral inflammation inhibits spinal learning (Ferguson et al., 2006) and, like the deficit observed after uncontrollable shock, this effect was reversed by the opioid antagonist naltrexone (Hook, Huie, & Grau, 2007). Further, like central sensitization, the induction of the learning deficit can be blocked by pretreatment with an NMDAR antagonist (Ferguson et al., 2006). Interestingly, the link to inflammation and neuropathic pain again implicates kappa-opioids, for inflammation induces a lasting increase in the endogenous kappa-opioid dynorphin (Wagner, Terman, & Chavkin, 1993; Wang et al., 2001) and microinjection of a kappa agonist into the spinal cord (an intrathecal [i.t.] injection) inhibits behavior signs of neuropathic pain (Eliav, Herzberg, & Caudle, 1999; Ho, Mannes, Dubner, & Caudle, 1997).

The data reviewed above suggest that a kappa-opioid mediated process modulates spinal learning. Our prior work (Joynes & Grau, 2004) relied on selective opioid antagonists, demonstrating that an opioid ligand was essential (*necessary*) to the expression of the learning deficit. In the present paper, we substitute an opioid agonist for intermittent shock to examine whether a kappa-opioid is *sufficient* to inhibit instrumental learning. We show that an opioid agonist (GR89696) that acts at the kappa-2 receptor inhibits learning in a dose-dependent fashion (Experiments 1 and 2). Further, GR89696 had no effect on instrumental performance (Experiment 3) and its effect on learning was naltrexone-reversible (Experiment 4). The effect of GR89696 waned within 24 h (Experiment 5) and drug treatment blunted the consequences of uncontrollable stimulation (Experiment 6). As a result of these findings, we suggest that the up-regulation of kappa-opioid activity may serve an adaptive function designed to limit NMDAR-dependent plasticity within the spinal cord.

2. General methods

2.1. Subjects

All protocols were approved by the Animal Care and Use committee at Texas A&M University. Male Sprague–Dawley rats obtained from Harlan (Houston, TX) served as subjects. Animals were approximately 100–120 days old and weighed between 360 and 460 g. Subjects were maintained on a 12 h light-dark schedule and housed individually. Food and water were available *ad libitum*, and behavioral testing was performed during the light portion of the cycle.

2.2. Surgery

Subjects were anesthetized with pentobarbital (50 mg/kg, i.p.), and the area surrounding the shoulders was shaved and sterilized with iodine. An anterior–posterior incision, approximately 1.5 cm long, was made over the 2nd thoracic vertebra (T2). The tissue immediately anterior to T2 was then cleared, and the exposed spinal cord was transected using cauterization. The resulting space was filled with Gelfoam (Harvard Apparatus, Holliston, MA), and a cannula consisting of 25 cm of polyethylene tubing (PE-10, VWR International, Bristol, CT) fitted with a stainless steel wire