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Noradrenaline increases pain facilitation from the brain during inflammatory pain

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ABSTRACT

Antidepressants that inhibit the recapture of noradrenaline have variable effects in chronic pain which may be related to the complex role of noradrenaline in pain modulation. Whereas at the spinal cord noradrenaline blocks nociceptive transmission, both antinociception and pronociception were reported after noradrenaline release in the brain. To study the role of noradrenaline in pain modulatory areas of the brain, we elected the dorsal reticular nucleus (DRt), a key pain facilitatory area located at the medulla oblongata. Three studies were performed. First, we show that the infusion in the DRt of nomifensine, which increases local extracellular levels of noradrenaline as shown by *in vivo* microdialysis, also enhances pain behavioral responses during both phases of the formalin test, a classic inflammatory pain model. Then, we demonstrate that the formalin test triggers the release of noradrenaline in the DRt in a biphasic pattern that matches the two phases of the test. Finally, we show that reducing noradrenaline release into the DRt, using an HSV-1 vector which decreases the expression of tyrosine hydroxylase in noradrenergic DRt-projecting neurons, attenuates pain behavioral responses in both phases of the formalin test. The increased noradrenaline levels induced by the infusion of nomifensine at the DRt, along with the hyperalgesic effects of noradrenaline released at the DRt upon noxious stimulation, indicates that noradrenaline may enhance pain facilitation from the brain. It is important to evaluate if antidepressants that inhibit noradrenaline recapture enhance pain facilitation from the brain herein attenuating their analgesic effects.

1. Introduction

Noradrenaline plays a key role in the inhibition of nociceptive transmission at the spinal cord level. Noradrenaline also controls the activity of pain modulatory centers of the brain, namely the rostral ventromedial medulla (Bie et al., 2003; Clark and Proudfit, 1991), the locus coeruleus (Sawamura et al., 2000) and the amygdala (Ortiz et al., 2007). However, contrary to the spinal cord, the effects of noradrenaline in the brain are complex, depending on the pain control area and the adrenoreceptors (ARs) involved. For example, noradrenaline mediates stress-induced analgesia in the amygdala via activation of α2-ARs (Ortiz et al., 2008) and opioid withdrawal hyperalgesia in the rostral ventromedial medulla via activation of α1-ARs (Bie et al., 2003). Understanding the effects of noradrenaline is important since antidepressants that inhibit noradrenaline reuptake are frequently used in chronic pain treatment and their analgesic action has been ascribed to the inhibitory effects of noradrenaline upon nociceptive transmission at the spinal cord (Attal et al., 2010; Bannister et al., 2009; Dharmshaktu et al., 2012; McCleane, 2008; Sawynok et al., 2001; Sindrup et al., 2005). However, systemic or oral administration of antidepressants which inhibit noradrenaline reuptake also increases noradrenaline levels in the brain, namely at the striatum (Gobert et al., 2004) and the hippocampus (Bloms-Funke et al., 2011; Tzschentke et al., 2007), which demands to study the effects of noradrenaline in pain control areas of the brain.

The dorsal reticular nucleus (DRt) plays a unique role in the facilitation of pain transmission at the spinal cord (Almeida et al., 2006; Heinricher et al., 2009; Lima and Almeida, 2002). The DRt is reciprocally connected with the spinal dorsal horn through a direct and putative excitatory pathway. This reverberating circuit allows enhancement of descending pain facilitation from the DRt in

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response to local arrival of nociceptive input (Lima and Almeida, 2002). The DRt receives noradrenergic input from several noradrenergic cell groups in the brainstem, namely the locus coeruleus (Almeida et al., 2002). We recently showed that during chronic neuropathic pain decreasing noradrenaline at the DRt reversed neuropathic pain (Martins et al., 2010) suggesting that noradrenaline may affect pain modulation from the DRt. It is unknown if the application of a noxious stimulus to the periphery triggers noradrenaline release in the DRt, herein affecting descending pain modulation.

We performed a study aiming to evaluate the mechanisms of noradrenergic control of pain modulation from the DRt. First we studied the effects of increasing noradrenaline levels in the DRt by local infusion of nomifensine, which inhibits the recapture of noradrenaline and dopamine (Katz et al., 2010; Kawahara et al., 1999; Tatsumi et al., 1997). Since the DRt does not contain dopaminergic neurons and is scarcely innervated by dopaminergic fibers (Almeida et al., 2002; Gasbarri et al., 1997) and nomifensine has previously shown to fail to increase dopamine levels in neuronal circuits with similar neurochemical arrangement (Kawahara et al., 1999), the effects of nomifensine can probably be ascribed to the noradrenaline increase at the DRt. We used *in vivo* microdialysis to quantify the increases of extracellular levels of noradrenaline at the DRt and evaluated the effects of nomifensine infusion on pain behavioral responses by the well-established formalin pain test which produces a characteristic biphasic behavioral response (Tjolsen et al., 1992). The first phase of the formalin test, also named acute phase, occurs within seconds after formalin injection and lasts for 10 min as a direct consequence of sensory afferents activation (McNamara et al., 2007). The second phase, also named tonic phase, starts 20 min after formalin injection and lasts about 30 min as a result of increased primary afferent drive with subsequent sensitization of spinal neurons (Pezet et al., 2008; Yashpal et al., 2001). Since the formalin model allows characterizing spontaneous pain and sensory abnormalities, which are major features of neuropathic pain (Finnerup and Jensen, 2006), it is very common to use the formalin test to evaluate the analgesic efficacy of several drugs, namely antidepressants (Nayebi et al., 2001; Munro, 2009; Otsuka et al., 2001). We also used *in vivo* microdialysis to evaluate whether formalin injection could trigger the release of noradrenaline in the DRt and to correlate the pattern of behavioral responses in the formalin test with the release of that neurotransmitter. Although, as discussed above, dopamine is unlikely to have an effect at the DRt, we also measured the levels of this catecholamine at the DRt during the formalin test. We also studied the effects of decreasing noradrenaline release in the DRt in the behavioral responses of the formalin test, using an HSV-1 vector carrying the tyrosine hydroxylase (TH) promoter, the activity of which is restricted to catecholaminergic cells (Song et al., 1997), and the cDNA for the TH enzyme in antisense orientation (Martins et al., 2010). Finally, we studied the involvement of α-adrenoreceptors in pain control from the DRt.

2. Material and methods

2.1. Animals

Male Wistar rats (Harlan colony, Zeist, The Netherlands) were maintained on a standard 12/12 h light/dark cycle and were provided with food and water *ad libitum*. The animals were acclimated to the housing facility for at least one week before any treatment. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of the College of Mathematics and Natural Sciences of the University of Groningen and were performed according to the ethical guidelines for pain investigation (Zimmermann, 1983).

2.2. Stereotaxic procedures

Rats weighing 285–315 g were anesthetized with isoflurane and placed on a stereotaxic frame for cannula implantation or injection of viral vectors into the left DRt. At the end of the procedure the animals received 0.1% finadyne (0.1 ml/kg, i.p.) for post-operative analgesia.

2.2.1. Cannula implantation

A guide cannula was implanted in the DRt for microdialysis ($n = 29$) or pharmacological ($n = 42$) experiments. The coordinates to target the left DRt were determined according to the rat brain atlas (Paxinos and Watson, 1998) relative to the interaural line (AP: −6.0 mm; LM: −1.4 mm; DV: −1.5 mm). The stainless steel guide cannula used for microdialysis was lowered until its tip was 1 mm above the DRt. For pharmacological experiments, the guide cannula was lowered until its tip was 3 mm above the DRt.

2.2.2. Viral vector injections

Two recombinant HSV-1 vectors, named THZ (control vector) and THa, constructed as described previously (Martins et al., 2010), were used to selective target noradrenergic neurons. Briefly, the THa vector, designed to knock down expression of TH through RNA interference, contains a cassette with the rat TH promoter and the full length human TH cDNA in reverse orientation relative to the promoter inserted in the HSV-1 thymidine kinase gene. The THZ vector is similar to the THa vector but contains the *lacZ* cDNA, in sense orientation, in place of the TH cDNA.

The vectors were used at 4×10^6 pfu/μl and were slowly injected (flow rate: 0.1 μl/2 min) in two rostrocaudal parts of the left DRt following the coordinates of the atlas Paxinos and Watson (1998) (first injection: AP:−6.0 mm; LM: −1.4 mm; DV: −1.5 mm; second injection: AP: −6.4 mm; LM: −1.3 mm; DV: −1.7 mm) with 1 μl injected per site. At the completion of the injections, the needle was left in place for 10 min before being slowly removed. Twenty-two days after injection, which is the time of maximal behavioral effects of THa in neuropathic pain due to the decrease of noradrenaline release at the DRt (Martins et al., 2010), the animals were divided into 3 groups. The first group consisted of animals injected with THZ ($n = 2$) and was used to evaluate the noradrenergic nature of the transduced neurons. The second group consisted of animals injected with THZ ($n = 6$) or THa ($n = 5$) and received an implantation of a guide cannula at the DRt, as above, for microdialysis. The third group consisted on animals injected with THZ ($n = 6$), THa ($n = 5$) or the equivalent volume of a vehicle solution (Veh; $n = 6$) and was used to perform the formalin test.

2.3. Formalin test

The animals were handled daily during the week before the formalin test, for habituation purposes (Tjolsen et al., 1992). This test is characterized by an acute phase (phase 1) and a tonic (phase 2), interposed by a quiescent period (interphase) (Tjolsen et al., 1992). Fifty microliters of 5% formalin were injected subcutaneously into the dorsal surface of the left hind paw using a 27-gauge needle. Animals were then placed on a clear Plexiglas chamber and the number of paw jerks was recorded, as previously described (Martins et al., 2011) on video tape during 5 min epochs for 60 or 90 min, depending on the purpose of the studies (60 min for pharmacological experiments and 90 min for microdialysis experiments).

2.4. Microdialysis experiments

In vivo microdialysis was performed to evaluate noradrenaline levels in the DRt in basal conditions following infusion of nomifensine or administration of viral vectors. During the formalin test we evaluated both noradrenaline and dopamine levels.

2.4.1. During nomifensine infusion

One week after stereotaxic surgery ($n = 6$), the stylet was replaced with a 2 mm open length probe (molecular weight cutoff 45–50 kDa; Brainlink BV, Groningen, The Netherlands) and following 3 h of Ringer's solution (140.0 mM NaCl; 4.0 mM KCl; 1.2 mM $CaCl_2$; 1.0 mM $MgCl_2$) perfusion at a flow rate of 2.0 μl/min, 4 microdialysate samples were collected in 15 min intervals. Nomifensine at a concentration of 10 μM was then added to the Ringer's solution and after 45 min of stabilization, 4 additional microdialysates samples were collected in 15 min intervals into the sample loop of an HPLC. After the last sample had been collected the probes continued to be perfused with Ringer's supplemented with nomifensine, and the rats received a subcutaneous injection of 50 μl of formalin at 5% into the dorsal surface of the left hind paw. In another group of animals ($n = 6$), the probes were perfused with Ringer's solution for 3 h and continued to be perfused with Ringer's after formalin injection, as above. The number of paw jerks was evaluated simultaneously, for 60 min after formalin injection.

Noradrenaline was quantitated by HPLC with electrochemical detection. The isocratic mobile phase (4.1 g sodium acetate, 210 mg 1-octanesulfonic acid, 186,12 mg EDTA and 10% of methanol in 1000 ml ultrapurified water, pH = 4) was delivered at 1.0 ml/min by a Shimadzu LC-10AD pump (Kyoto, Japan) onto a C18 reversed-phase column (150 × 4.6 mm; 3 μm particle size; Waters Corporation, Milford, USA). The oxidizing potential of the electrochemical cell was set at +300 mV, the reduction potential to −300 mV.

2.4.2. During the formalin test

One week after stereotaxic surgery, the stylet was replaced with a 2 mm open length microdialysis probe (molecular weight cutoff 45–50 kDa; Brainlink BV,