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BRAIN RESEARCH

Research Report

Contribution of the rostral ventromedial medulla to post-anxiety induced hyperalgesia

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ABSTRACT

Rats exposed to an elevated plus maze (EPM) with four open arms display antinociception while on the maze and hyperalgesia immediately upon removal. Little is known about the neural mechanisms underlying EPM-induced antinociception and the subsequent hyperalgesia except that the antinociception is not mediated by endogenous opioids. The objective of the present study was to test the hypothesis that endogenous cannabinoids and/or the rostral ventromedial medulla (RVM) contributes to EPM-induced antinociception. Administration of the CB1 receptor antagonist AM251 (1 mg/kg, i.p.) had no effect on baseline nociception to formalin administration into the hindpaw or on the antinociception produced by placing a rat on the open EPM. Likewise, inactivation of the RVM by microinjecting the $GABA_A$ receptor agonist muscimol (10 ng/0.5 μL) had no effect on the antinociceptive effect of placing a rat in the EPM. However, RVM inactivation blocked the hyperalgesia produced upon removal from the EPM. Although distinct classes of RVM neurons inhibit and facilitate nociception, the present data demonstrate that the antinociception induced by the EPM and the subsequent hyperalgesia is mediated by distinct neural pathways.

1. Introduction

A variety of threatening and/or stressful stimuli have been shown to produce antinociception (Fanselow, 1991; Fardin et al., 1984; Kelly, 1982; Terman, et al., 1984; Watkins and Mayer, 1982). Exposure to the standard elevated plus-maze (EPM, two enclosed and two open arms), a test originally validated to study anxiety-like behaviors in rats and mice (e.g., Lister, 1987; Pellow et al., 1985; Stephens et al., 1986), also has been shown to produce antinociception (Lee and Rodgers, 1990, 1991; Rodgers et al., 1992). This antinociception was relatively mild, but persisted for up to 30 min following removal from the EPM (Lee and Rodgers, 1990, 1991). On the other hand, it has been shown that removal of the walls (so all four arms of the maze are open) enhances the antinociceptive effects (Cornélio and Nunes-de-Souza, 2009; Mendes-Gomes and Nunes-de-Souza, 2005, 2009). Unlike many aversive stimuli (e.g., footshock) (Terman et al., 1984) and studies with standard EPM (Lee and Rodgers, 1990, 1991), EPM-induced antinociception is short lived: Removing rats from the maze causes an immediate shift from antinociception to hyperalgesia (Cornélio et al., 2011). Little is known about the

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Abbreviations: EPM, elevated plus maze; RVM, rostral ventromedial medulla; PAG, periaqueductal gray

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neural mechanisms underlying EPM-induced antinociception or the subsequent hyperalgesia.

Previous research has shown that endogenous opioids do not contribute to EPM-induced antinociception. This antinociception is not reversed by the opioid receptor antagonist naltrexone and does not produce cross-tolerance to morphine antinociception (Cornélio and Nunes-de-Souza, 2009). Many other transmitter systems could underlie EPM induced antinociception. Endogenous cannabinoids are a likely candidate because both cannabinoids and exposure to the EPM are associated with anxiety (Ruehle et al., 2012) and produce antinociception (Hohmann et al., 2005; Mendes-Gomes and Nunes-de-Souza, 2005; Pertwee, 2001).

Endogenous cannabinoids and cannabinoid receptors are present at several levels of the pain pathway, from peripheral sensory nerve endings to spinal cord and supraspinal centers (Iversen, 2003). Synthetic and endogenous cannabinoids have antinociceptive and anti-hyperalgesic effects in a variety of animal models of acute and tonic pain when administered orally, systemically or directly into brain or spinal cord (for review see Pertwee, 2001). In addition, endocannabinoids have been shown to contribute to some forms of stress-induced analgesia such as that elicited by brief and continuous electric foot shock to rats (Hohmann et al., 2005).

The antinociceptive effects of opioids and cannabinoids are known to be mediated in part by the nociceptive modulatory system that runs through the periaqueductal gray (PAG) and rostral ventromedial medulla (RVM) (Hohmann et al., 2005; Lane et al., 2005; Meng et al., 1998; Proudfit and Anderson, 1975; Yaksh et al., 1977). The RVM is of particular interest because RVM on- and off-cells (Fields et al., 1983) have been shown to facilitate and inhibit nociception, respectively (Heinricher et al., 1994; Neubert et al., 2004). These findings suggest that endogenous cannabinoids could mediate EPM-induced antinociception, and the RVM could contribute to both antinociception and post-EPM hyperalgesia. These hypotheses were tested by exposing rats to the EPM following systemic administration of the CB1 receptor antagonists AM251 or inactivation of the RVM with the $GABA_A$ receptor agonist muscimol.

2. Results

2.1. *Experiment 1: endogenous cannabinoids*

Systemic administration of the CB1 receptor antagonist AM251 had no effect on baseline nociception assessed during the first phase of the formalin test [t (26) = 0.41; $p > 0.05$]. The average time spent licking the hindpaw following formalin administration was similar whether rats were pretreated with AM251 (52.9 ± 5.5 s) or vehicle (56.4 ± 6.4).

As reported previously (Cornélio and Nunes-de-Souza, 2009), exposure to the open EPM during the second phase of the formalin test produced a significant antinociception compared to rats in the enclosed EPM [Fig. 1; $F(1,24) = 52.75$, $p < 0.05$]. This antinociception was not reversed by AM251 administration as indicated by the lack of an interaction between rats pretreated with AM251 or vehicle ($F(1,24) = 0.28$, $p = 0.60$). Although administration of AM251 did not produce a statistically significant difference in the amount of time spent licking the hindpaw compared to vehicle treated rats ($F(1,24) = 2.68$, $p = 0.11$), there was a trend toward greater antinociception in rats treated with AM251. Enhanced antinociception on the EPM would be consistent with the anxiogenic effects of AM251 (Ruehle et al., 2012; Sink et al., 2010).

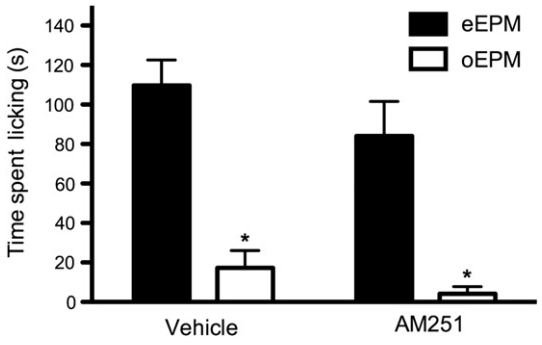

Fig. 1 – Endogenous cannabinoids do not contribute to EPM-induced antinociception. Time (in seconds) spent licking the paw during the second phase (n = 7/group) of the formalin test in rats treated with vehicle or AM251 (1 mg/kg, i.p.) and exposed to the enclosed EPM or open EPM. Although exposing rats to the open EPM produced antinociception compared to rats in the enclosed EPM (* denotes a significant difference, $p < 0.05$), administration of AM251 had no effect. Note: animals were exposed to the enclosed EPM or open EPM only during the second phase of the formalin test.

2.2. *Experiment 2: rostral ventromedial medulla*

A total of 39 rats had cannula placements in or along the border of the RVM (Fig. 2). The placements were similar whether rats were injected with saline into the RVM and placed in the open (N = 8) or enclosed EPM (N = 9), or injected with muscimol and placed in the open (N = 12) or enclosed EPM (N = 10).

The first phase of the formalin test was assessed prior to the RVM microinjection. Thus, the groups did not differ at this point and there was no difference in the amount of time spent licking the hindpaw between the saline (51.8 ± 3.2 s) and muscimol (47.5 ± 4.5 s) treated groups during this phase [t (37) = 0.74; $p > 0.05$].

Rats were placed in the EPM 15 min after saline or muscimol was microinjected into the RVM. Rats exposed to the open EPM spent significantly less time licking the hindpaw during the second phase of the formalin test compared to rats in the enclosed EPM [$F(1,35) = 6.84$, $p < 0.05$]. Inactivation of the RVM by microinjecting muscimol into the RVM had no effect on this antinociception [$F(1,35) = 0.00$, $p > 0.05$; Fig. 3] indicating that the RVM does not contribute to EPM-induced antinociception.

Assessment of nociception using the hot plate test immediately after removing the rat from the open EPM produced a hot plate latency of 10.4 ± 1.0 s (Fig. 4). Microinjection of muscimol into the RVM reversed this hyperalgesia as is evident by a significant increase in hot plate latency [t (11) = 2.275, $p < 0.05$]. Closer analysis of this effect revealed a bimodal effect of muscimol. Six rats injected with muscimol and removed from the open EPM had hot plate latencies greater than 18 s and 6 had