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Calcium/calmodulin-dependent protein kinase IV mediates acute nicotine-induced antinociception in acute thermal pain tests

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Abstract

Calcium activated second messengers such as calcium/calmodulin-dependent protein kinase II have been implicated in drug-induced antinociception. The less abundant calcium activated second messenger, calcium/calmodulin-dependent protein kinase IV (CaMKIV), mediates emotional responses to pain and tolerance to morphine analgesia; however its role in nicotine-mediated antinociception is currently unknown. The goal of this study was to evaluate the role of CaMKIV in the acute effects of nicotine, primarily acute nicotine-induced antinociception. CaMKIV knockout (−/−), heterozygote (+/−), and wild-type (+/+) mice were injected with various doses of nicotine and evaluated in a battery of tests, including the tail-flick and hot-plate tests for antinociception, body temperature, and locomotor activity. Our results show a genotype-dependent reduction in tail-flick and hot-plate latency in CaMKIV (+/−) and (−/−) mice after acute nicotine treatment, while no difference was observed between genotypes in the body temperature and locomotor activity assessments. The results of this study support a role for CaMKIV in acute nicotine-induced spinal and supraspinal pain mechanisms, and further implicate involvement of calcium-dependent mechanisms in drug-induced antinociception.

Keywords

nicotine; calcium/calmodulin-dependent protein kinase IV; antinociception; tail-flick; hot-plate, mouse

Introduction

Nicotine-induced activation of neuronal nicotinic receptors (nAChRs) results in increased permeability to Na⁺ and Ca²⁺ (Mulle et al., 1992), and a subsequent increase in intracellular calcium. Downstream activation of the calcium-mediated second messengers, calcium/calmodulin-dependent protein kinase II (CaMKII) and IV (CaMKIV) is implicated in mediating drug-induced pain mechanisms. Several studies also implicate a role for nicotinic acetylcholine receptors (nAChRs) in pain mechanisms (Damaj et al., 1998; Marubio et al., 1999; Decker et al., 2004; Simons et al., 2005). Indeed, nicotine induces potent antinociception in various rodent pain models (Han et al., 2005; Campbell et al., 2006; Damaj et al., 2007c; Galeote et al., 2008). Interestingly, CaMKII is activated in the mouse

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spinal cord via $\beta 2$ -containing nAChRs after an acute systemic or intrathecal injection of nicotine (Damaj, 2000; Damaj, 2007a), and after chronic nicotine treatment (Damaj, 2005). Behaviorally, CaMKII mediates expression and development of nicotine-induced antinociception in mice (Damaj, 2005). These findings support the involvement of CaMKII in nicotine-induced pain response.

Similarly, CaMKIV dependent signaling pathways mediate emotional responses to pain (Ko et al., 2005), and analgesic tolerance to morphine, via a cyclic AMP response element binding protein (CREB) signaling mechanism (Ko et al., 2006). To date, the role of CaMKIV in nicotine-induced antinociception is unknown. Thus, the goal of the current study was to investigate CaMKIV in the acute behavioral effects of nicotine, in particular acute nicotine-induced antinociception. Given that CaMKII was shown to play an important role in nicotine-induced antinociception, we hypothesized that CaMKIV is also an important molecular component mediating the acute pharmacological effects of nicotine. To test this hypothesis, we measured tail-flick, hot-plate, body temperature, and locomotor activity following nicotine injection in CaMKIV wild-type (+/+), heterozygote (+/-), and knockout (-/-) mice.

Methods

Subjects

Male and female breeders null for the CaMKIV gene and +/+ mice were purchased from Jackson Laboratories (Bar Harbor, ME) and bred in an animal care facility at Virginia Commonwealth University. Mutant and +/+ mice were obtained from crossing CaMKIV +/- mice. Male mice were used for all studies and were 8-10 weeks of age at the start of all studies. Animals were maintained in an Association for Assessment and Accreditation of Laboratory Animal Care-approved animal care facility and the studies were approved by the Institutional Animal Care and Use Committee of Virginia Commonwealth University.

Acute nicotine assessment

Naïve male CaMKIV -/-, +/- mice and +/+ littermates were injected s.c. with various doses of nicotine. Antinociception using the tail-flick and hot-plate tests, rectal temperature and locomotor activity were measured as previously described (Jackson et al., 2009). Doses selected for each assay reflect the ED₂₀ and ED₈₀ values of the nicotine dose response curves for each response (Jackson et al., 2010). Each group contained 6-8 mice per treatment.

In brief, antinociception was assessed by the tail-flick and hot plate methods. In the tail-flick test, mice were tested 5 min after injection of 1 or 2.5 mg/kg nicotine. A control response was measured for each mouse, followed by a test for latency after drug treatment. The amount of time taken for each animal to remove the tail from the heat source was recorded. A maximum latency of 10 s was implemented to minimize tissue damage. In the hot plate test, mice were tested 2 h before and 5 min after injection of 1 or 2.5 mg/kg nicotine. Mice were placed into a glass cylinder with a 10 cm diameter on a hot-plate (Thermojust Apparatus) maintained at 55.0 °C. The reaction time was scored when the animal began to exhibit signs of pain avoidance such as jumping or paw licking. Animals that did not respond to the noxious heat stimulus after 40 s were removed from the plate. Tail-flick and hot-plate antinociceptive responses were calculated as %MPE, where % MPE = [(test – control)/(10 – control) × 100] or where % MPE = [(test – control)/(40 – control)] × 100 respectively.

Rectal temperature was measured by a thermistor probe (inserted 24 mm) and digital thermometer (YSI Inc., Yellow Springs, OH). Readings were taken just before and at 30 min after injection of 1 or 2.5 mg/kg nicotine. The difference in rectal temperature ($\Delta^{\circ}\text{C}$) before and after treatment was calculated for each mouse. For motor activity testing, mice were placed into individual Omnitech photocell activity cages (28×16.5 cm) 5 min after administration of saline, 0.5, or 1.5 mg/kg nicotine. For 30 min, interruptions of the photocell beams were recorded. Data are expressed as the average number of photocell interruptions during the 30 min test period.

Drugs

(–)-Nicotine hydrogen tartrate salt was purchased from Sigma-Aldrich Inc. (St. Louis, MO, USA). Nicotine was dissolved in physiological saline (0.9% sodium chloride) at a volume of 10 ml/kg body weight and was administered subcutaneously (s.c.). Doses are expressed as free base of the drug.

Statistical analysis

Statistical analyses of all studies were performed using a two-way analysis of variance with treatment and genotype as between subject factors with the Statview® software. The Student Newman-Kuels posthoc test was used to further analyze significant interactions. All differences were considered significant at $p < 0.05$.

Results

Antinociceptive effects of nicotine in the tail-flick and hot-plate tests are shown in Figures 1A and B respectively. Significant main effects of dose (tail-flick: $F_{(2,45)} = 21.53$, $p < 0.001$; hot-plate: $F_{(2,45)} = 22.21$, $p < 0.001$), genotype (tail-flick: $F_{(2,45)} = 9.71$, $p < 0.001$; hot-plate: $F_{(2,45)} = 8.28$, $p < 0.001$), and a significant dose $\times$ genotype interaction (tail-flick: $F_{(4,45)} = 5.05$, $p < 0.05$; hot-plate: $F_{(4,45)} = 5.16$, $p < 0.001$) were found in both assessments. Nicotine significantly increased tail-flick and hot-plate latency in CaMKIV $+/+$ mice. A significant reduction of the responses was observed in the CaMKIV $+/-$ mice, and a total elimination of the responses was observed in CaMKIV $-/-$ mice after administration of the higher nicotine dose (2.5 mg/kg), but not the lower dose (1 mg/kg). Baseline latencies in the hot-plate and tail-flick tests did not differ between genotypes at either nicotine dose.

In the body temperature assessment, nicotine significantly decreased body temperature in all genotypes (significant main effect of dose: $F_{(2,45)} = 93.49$, $p < 0.001$); however, there were no significant main effect of genotype or dose $\times$ genotype interaction, indicating that CaMKIV $+/-$ and $-/-$ mice did not differ from their $+/+$ counterparts at either nicotine dose in this assessment (Figure 2A). Basal body temperature values did not differ between genotypes. Similarly, in the locomotor activity assessment, shown in Figure 2B, nicotine reduced activity in all genotypes (significant main effect of dose: $F_{(2,63)} = 119.89$, $p < 0.001$, no significant main effect of genotype or dose $\times$ genotype interaction), though CaMKIV $+/-$ and $-/-$ mice did not differ from $+/+$ counterparts in the saline or nicotine groups.

Discussion

The goal of the current study was to investigate the role of CaMKIV in the acute behavioral effects of nicotine, in particular acute nicotine-induced antinociception. The results implicate a role for CaMKIV-dependent signaling in nicotine-induced antinociception in mice. This involvement of CaMKIV did not extend to other acute effects of nicotine since no significant differences between genotypes were observed in either the body temperature or locomotor activity assessment after nicotine treatment. Because responses to the hot-plate

are thought to be largely centrally mediated, whereas the tail-flick is considered primarily a spinal reflex (Caggiula et al., 1995), we propose that our results support a role for CaMKIV in both nicotine-induced spinal and supraspinal antinociceptive mechanisms.

In previous studies, CaMKII mediated the tail-flick, but not hot-plate responses, suggesting CaMKII involvement in nicotine-induced spinal, but not supraspinal antinociception (Damaj, 2007b). In the current assessment, we found CaMKIV involvement in both spinal and supraspinal mechanisms of nicotine-induced antinociception, suggesting that supraspinal nicotine-induced pain mechanisms involve CaMKIV to a greater extent than CaMKII. One possible target is CREB, a transcription factor phosphorylated by CaMKIV at Ser133 in the nuclei of neurons (Soderling, 1999; Deisseroth and Tsien, 2002). CREB phosphorylation is relevant to the development of tolerance to the analgesic effects of morphine (Ko et al., 2006). CREB phosphorylation at Ser133 was also increased in spinal cord neurons after formalin injection into the hind paw (Ji and Rupp, 1997). Additionally, phosphorylated CREB (pCREB) was upregulated in spinothalamic tract cells and implicated in central neuropathic pain (Crown et al., 2005). These studies further implicate CREB as a potential downstream target of CaMKIV-mediated pain responses. CaMKII can also phosphorylate CREB (Dash et al., 1991; Sheng et al., 1991); however, CaMKII does not enter the nucleus as CaMKIV does (Matthews et al., 1994), and as a result may not regulate calcium-mediated changes in gene expression associated with nicotine-induced supraspinal pain mechanisms. Interestingly, CaMKIV does not contribute to mechanisms of acute or persistent pain after tissue injury or inflammation under basal conditions (Wei et al., 2002), suggesting a role for CaMKIV in certain pain mechanisms specifically under drug induced conditions.

Overall, our results indicate that CaMKIV mechanisms mediate acute nicotine-induced spinal and supraspinal antinociception in the mouse. To our knowledge, the current study provides the first evidence of a role for CaMKIV in acute nicotine-induced antinociception and further implicates the involvement of calcium-dependent mechanisms in nAChR-mediated pain responses.

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Figure 1. Acute nicotine-induced antinociception is reduced in CaMKIV $-/-$ mice in a genotype-dependent manner

Mice were injected s.c. with 1 and 2.5 mg/kg nicotine (s.c.) and antinociception via the tail-flick and hot-plate were assessed. Nicotine induced a significant increase in (A) the tail-flick and (B) hot-plate latency in CaMKIV $+/+$ mice; however a genotype-dependent reduction in latency was observed after 2.5 mg/kg nicotine in CaMKIV $+/-$ and $-/-$ mice. Each point represents the mean + S.E.M. of 6-8 mice per group. * $p < 0.05$ vs. saline; ** $p < 0.05$ vs. saline and 1 mg/kg nicotine; + $p < 0.05$ vs. CaMKIV $+/+$ mice; ++ $p < 0.05$ vs. CaMKIV $+/+$ and $+/-$ mice.

Figure 2. Acute nicotine-induced hypothermia and hypomotility are preserved in CaMKIV $-/-$ mice

(A) Nicotine induced a significant hypothermia response in CaMKIV +/+, +/-, and -/- mice with no significant differences between genotypes. (B) Nicotine decreased locomotor activity in CaMKIV +/+, +/-, and -/- mice with no significant difference between genotypes. Each point represents the mean $\pm$ SEM for 6-8 mice per group. * significant main effect of treatment, $p < 0.05$.