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ROLE OF PREFRONTAL CORTICAL CALCIUM-INDEPENDENT PHOSPHOLIPASE A_2 IN ANTINOCICEPTIVE EFFECT OF THE NOREPINEPHRINE REUPTAKE INHIBITOR ANTIDEPRESSSANT MAPROTILINE

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Abstract—The prefrontal cortex is essential for executive functions such as decision-making and planning. There is also accumulating evidence that it is important for the modulation of pain. In this study, we investigated a possible role of prefrontal cortical calcium-independent phospholipase A_2 ($iPLA_2$) in antinociception induced by the norepinephrine reuptake inhibitor (NRI) and tetracyclic (tricyclic) antidepressant, maprotiline. Intraperitoneal injections of maprotiline increased $iPLA_2$ mRNA and protein expression in the prefrontal cortex. This treatment also reduced grooming responses to von-Frey hair stimulation of the face after facial carrageenan injection, indicating decreased sensitivity to pain. The antinociceptive effect of maprotiline was abrogated by $iPLA_2$ antisense oligonucleotide injection to the prefrontal cortex, indicating a role of this enzyme in antinociception. In contrast, injection of $iPLA_2$ antisense oligonucleotide to the somatosensory cortex did not reduce the antinociceptive effect of maprotiline. Lipidomic analysis of the prefrontal cortex showed decrease in phosphatidylcholine species, but increase in lysophosphatidylcholine species, indicating increased PLA2 activity, and release of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) after maprotiline treatment. Differences in sphingomyelin/ceramide were also detected. These changes were not observed in maprotiline-treated mice that received $iPLA_2$ antisense oligonucleotide to the prefrontal cortex. Metabolites of DHA and EPA may help to strengthen a known supraspinal antinociceptive pathway from the prefrontal cortex to the periaqueductal gray. Together, results indicate a role of prefrontal cortical $iPLA_2$ and its enzymatic products in the antinociceptive effect of maprotiline.

Key words: noradrenaline, prefrontal cortex, antinociception, lipid mediators, pain.

INTRODUCTION

The prefrontal cortex is essential for executive functions such as decision-making, planning and working memory (Fuster, 2008). More recently, there is also accumulating evidence that the prefrontal cortex plays an important role in the modulation of pain (Loggia et al., 2015). Functional neuroimaging studies show increased prefrontal cortical activity during anticipation of analgesia with placebo treatment (Benedetti et al., 2005; Wager, 2005). The prefrontal cortex could have a role in pain modulation though its projections to the periaqueductal gray (PAG), thereby affecting the descending pain inhibitory pathway (Valet et al., 2004; Ohara et al., 2005; Xie et al., 2009). Electrical activation of prefrontal cortical fiber projections to the midbrain induces antinociceptive effects in rodents (Hardy and Haigler, 1985; Zhang et al., 1998). A close relationship also exists between pain and anxiety/depression that may involve the prefrontal cortex. Antidepressants, especially tricyclic antidepressants, are used in the management of orofacial pain, lower back pain, and neuropathic pain (Lynch and Watson, 2006; Saarto and Wiffen, 2010). Antidepressants that are effective in antinociception are usually those that act on the noradrenergic system (norepinephrine reuptake inhibitors, NRI), or the noradrenergic and serotoninergic systems (Serotonin-Norepinephrine Reuptake Inhibitors or SNRI), whereas those that act mostly on the serotoninergic system are generally less effective (Sansone and Sansone, 2008;

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Abbreviations: DHA, docosahexaenoic acid; EDTA, ethylenediaminetetraacetic acid; EPA, eicosapentaenoic acid; $iPLA_2$, calcium-independent phospholipase A_2; MRM, Multiple Reaction Monitoring; NRI, noradrenaline reuptake inhibitor; PAG, periaqueductal gray; PFC, prefrontal cortex; PVDF, polyvinylidene difluoride.

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Verdu et al., 2008). Currently, however, relatively little is known about the biological substrates of antidepressant-induced antinociception.

Maprotiline is an NRI-antidepressant with a tetracyclic structure that is closely related to tricyclic antidepressants. Besides its antidepressive properties, maprotiline has antinociceptive effects in conditions such as chronic lower back pain (Atkinson et al., 1999; Banafshe et al., 2015). It possesses strong noradrenaline reuptake inhibitor activity, and part of its antidepressive action has recently been linked to activity of an enzyme, calcium-independent phospholipase A_2 ($iPLA_2$) in the prefrontal cortex (Lee et al., 2012). $iPLA_2$ acts on brain glycerophospholipids to release docosahexaenoic acid (DHA) and lysophospholipids (Farooqui et al., 1999, 2006; Rapoport, 2013). It is highly expressed in the hippocampus, striatum and cerebral cortex (Ong et al., 2005; Lee et al., 2012), and brain imaging of $iPLA_2$ deficient mice shows reduced brain DHA metabolism and signaling (Basselin et al., 2010; Cheon et al., 2012). $iPLA_2$ plays a role in neurite outgrowth, during development and in response to injury (Schaeffer et al., 2009).

Our previous study has shown that maprotiline treatment causes significant increase in lysophosphatidylcholine levels and decrease in phosphatidylcholine levels, indicating increased PLA_2 activity, and endogenous release of long chain polyunsaturated fatty acids (PUFAs) such as DHA in the prefrontal cortex (Lee et al., 2012). Inhibition of $iPLA_2$ in the prefrontal cortex abrogated the changes in lipid profiles, and abolished the antidepressant-like effect of maprotiline during the forced-swim test in mice (Lee et al., 2012). Results suggest a role of prefrontal cortical $iPLA_2$ in the *antidepressive* effect of maprotiline (Lee et al., 2012). In this study, we investigate a possible role of prefrontal cortical $iPLA_2$ in the *antinociceptive* effect of maprotiline, using the mouse facial carrageenan injection model of inflammatory pain (Yeo et al., 2004; Poh et al., 2012). It is hoped that this would provide some insights into mechanisms of antinociceptive effect of antidepressants, and cortical areas involved in antinociception.

EXPERIMENTAL PROCEDURES

Animals

A total of 92 male C57BL mice weighing between 20 and 30 g each and around 6–8 weeks old were used throughout this study. They were obtained from NUS Comparative Medicine Animal Facility and were housed under defined conditions (65% relative humidity, 22 °C room temperature and 12 h of lighting daily) with unrestricted access to water and food. All mice were randomized to treatment (Fig. 1).

Animal welfare and ethical statement

All animal procedures were in compliance with national and international policies following the National Advisory Committee for Laboratory Animal Research (NACLAR) Guidelines. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of the National University of Singapore (NUS) under the IACUC protocol number 085/11 and were performed as humanely as possible.

Antidepressants

For the first part of the study to investigate the effects of maprotiline treatment on prefrontal cortical $iPLA_2$ expression, mice were given daily intraperitoneal (i.p.) injections of maprotiline or vehicle (saline) for the duration of the study before the prefrontal cortex was harvested for real-time RT-PCR and Western blot analyses. For the pain behavioral assay, 21-day pretreatment of maprotilline or saline was administered before oligonucleotide administration and carrageenan injection, and every day thereafter until the end of the experiment. The duration of maprotilline treatment has been shown in the literature to be sufficient for the antidepressant properties of maprotilline to be exhibited (Lee et al., 2012). The dose of 10 mg/kg and the route of administration chosen for maprotilline treatment was based on previous studies which showed both behavioral and neurochemical changes in animal models after administration (Parra et al., 2000; Tan et al., 2006; Miranda et al., 2013).

Real-time RT-PCR

Mice were deeply anesthetized with a ketamine/medetomidine solution and sacrificed by decapitation at the end of antidepressant treatment regime. The prefrontal cortex was removed and snap frozen in liquid nitrogen. RNA was extracted from the prefrontal cortex and reverse transcribed using High-Capacity cDNA Reverse Transcription Kits (Applied Biosystems, CA, USA). The conditions for the reverse transcription reactions were 25 °C for 10 min followed by 37 °C for 120 min and 85 °C for 5 min. Quantitative real-time PCR amplification was performed in the 7500 Real-time PCR system (Applied Biosystems, CA, USA) using the converted cDNA together with TaqMan® Universal PCR Master Mix (Applied Biosystems, CA, USA) and TaqMan® probes for mouse $iPLA_2\beta$ and β-actin (Mm01299491_m1 and #4331182 respectively, synthesized by Applied Biosystems). The β-actin probe was used as a reference for real-time RT-PCR. The conditions for real-time PCR amplification were initial incubation at 50 °C for 2 min and 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. All amplification reactions were carried out in triplicate. The number of reaction cycles at which the reporter fluorescence emission surpasses the preset threshold level is the threshold cycle, Ct. There is an inverse correlation between the Ct value and the level of target mRNA. Amplified transcripts were quantified using the comparative Ct method with the formula for relative fold change = $2^{-\Delta\Delta Ct}$ (Livak and Schmittgen, 2001). The mean and standard error for each treatment group were calculated, and possible significant differences analyzed using two-tailed Student's *t*-test. $P < 0.05$ was considered significant.