

Antidepressant-like effect of caffeic acid: Involvement of the cellular signaling pathways

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Caffeic acid is a phenolic compound widely distributed in plants and beverages such as coffee. Although its mechanism of action is poorly understood, caffeic acid reportedly induces antidepressant-like and neuroprotective effects. This study aimed to investigate the involvement of cellular signaling pathways in acute antidepressant-like effect induced by caffeic acid in mice. All procedures were approved by the Institutional Animal Ethics Committee of the UNIVALI n. 021/2013. Female *Swiss* mice were administered with vehicle, caffeic acid (5 mg/kg, p.o.), inhibitor (H-89, U0126, chelerythrine, or PD9859, i.c.v.) or caffeic acid plus inhibitor. The behavioral effects were evaluated 1h after the administration of compounds to mice using tail suspension test (TST) and open field test (OFT). The results showed that the antidepressant-like effect of caffeic acid in mice was possibly mediated by the activation of PKA, MEK 1/2, PKC and MAPK (as assessed using TST), without compromising their locomotor activity (as assessed using OFT). Our results demonstrated, at least in part, the pathways involved in the neuroprotective and behavioral effects of caffeic acid.

Keywords: Caffeic acid. MAPK. PKA. PKC. MEK.

INTRODUCTION

Major depressive disorder (MDD) affects approximately 364 million people worldwide and is the leading cause of suicide (WHO, 2017). The pharmacological treatment of MDD is limited, has excessive side effects, and is generally ineffective (Otte *et al.*, 2016). Inflammation and oxidative stress are events related to the neurobiological basis of MDD (Maes *et al.*, 2012). Interestingly, coffee has been studied as a promising adjunct in the treatment for depressive disorders (Tenore *et al.*, 2015). Considered as one of the most consumed beverages globally, coffee deserves attention for the range of positive results demonstrated in animal models of diabetes, Parkinson's disease, Alzheimer's disease, and depression. The metabolic effects of coffee are mediated

by its several bioactive constituents, particularly caffeine, chlorogenic acid, ferulic acid, and caffeic acid (Hall *et al.*, 2015; Kaster *et al.*, 2015).

In line with these assumptions, evidence suggests that caffeic acid, one of the most common phenolic compounds present in plants and beverages, such as coffee, can have beneficial effects on (i) pronounced increase in oxidative stress (Kalonía *et al.*, 2009b); (ii) neurotoxicity (Kalonía *et al.*, 2009a; Noelker *et al.*, 2005; Taram, Winter, Linseman, 2016); (iii) inflammatory process (Búfalo *et al.*, 2013); and (iv) anxiety (Takeda *et al.*, 2003); and depression (Takeda *et al.*, 2002; 2003). Interestingly, Takeda *et al.* (2003) demonstrated that caffeic acid triggers an antidepressant-like effect mediated by α_{1A} -adrenoreceptor. Additionally, this compound could treat depressive-like behavior of mice subjected to chronic stress model by modulating glucocorticoid receptors (Lee *et al.*, 2014).

However, the mechanism underlying the antidepressant effect of caffeic acid, besides the role of cellular signaling pathways in this effect, is not entirely

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known. Several reports have demonstrated the role of antidepressants in modulating protein kinases involved in cell survival. Long-term use of these drugs can directly activate cAMP-dependent protein kinase, protein kinase C (PKC), and the Ca^{2+} /calmodulin-dependent protein kinase II (CaMKII) pathway, as well as the mitogen-activated protein kinase/extracellular signal-regulated protein kinase (MAPK/ERK) pathway. Protein kinases also phosphorylate the cAMP response element-binding protein (CREB), increasing the expression of genes such as brain-derived neurotrophic factor (BDNF), which can modulate mood alterations (Castrén, Kojima, 2017; Niciu *et al.*, 2013; Taylor *et al.*, 2005). Thus, this experimental analysis aimed to investigate the modulation of cell signaling pathways triggered by caffeic acid administration to mice.

MATERIAL AND METHODS

Animals

This study was performed after receiving approval of the Institutional Ethics Committee (CEUA) of Universidade do Vale do Itajaí – UNIVALI, (No. 021/13). Female *Swiss* mice (25-30 g, 3 months) were used for the pharmacological assays. The animals were housed at a temperature of $22 \pm 2^\circ\text{C}$ under a 12:12h light:dark cycle (lights on at 6:30 a.m.), with free access to food and water.

Drugs and Treatments

Caffeic acid and fluoxetine (Sigma Chemical Co., St. Louis, USA) were dissolved in saline (vehicle) and administered orally (p.o.) by gavage at a volume of 10 mL/kg body weight. The inhibitors H-89 (N-(2-(4-Bromocinnamylamino)ethyl)-5-isoquinolinesulfonamide), U0126 (1,4-Diamino-2,3-dicyano-1,4-bis(o-aminophenylmercapto)butadiene monoethanolate), chelerythrine, and PD98059 (2-(2-Amino-3-methoxyphenyl)-4H-1-benzopyran-4-one) (Sigma Chemical Co., St. Louis, USA) were dissolved in saline with 1% DMSO and administered intracerebroventricularly (i.c.v.) (5 μl /site). I.c.v. administration was performed in mice under ether

anesthesia using a microsyringe (10 μl , Hamilton) connected to a 26-gauge stainless-steel needle that was inserted perpendicularly 2 mm deep through the skull according to the procedure originally described (Cunha *et al.*, 2014). A volume of 5 μl was administered into the left lateral ventricle. The injection was given over 30 s, and the needle was maintained at the injection location for another 30 s to avoid reflux of the injected drugs. The injection site was 1 mm to the right or left from the mid-point on a line drawn through to the anterior base of the ears.

The involvement of cellular signaling pathways in the antidepressant-like effect of caffeic acid was investigated through the preadministration of mice with the inhibitors H-89 (1 μg /site, i.c.v., PKA inhibitor), U0126 (5 μg /site, i.c.v., MEK 1/2 inhibitor), chelerythrine (1 μg /site i.c.v., PKC inhibitor), and PD98059 (5 μg /site, i.c.v., MAPKs inhibitor). 30 min after the pre-treatment, mice were administered with caffeic acid (5 mg/kg, p.o.) or vehicle, and within 60 min later, they were submitted to TST and OFT. Doses were selected in accordance with literature (Búfalo *et al.*, 2013; Cunha *et al.*, 2014; Hall *et al.*, 2015; Kalonia *et al.*, 2009a; Zeni *et al.*, 2012).

Tail suspension test (TST)

TST is widely used to evaluate compounds for their antidepressant potential. For this test, mice were individually suspended by the tail 50 cm above the floor, with acoustic and visual isolation. Immobility time was recorded for 6 min by a trained observer blinded to the experimental groups. Compounds with antidepressant effects increased agitation and decreased the immobility time. Immediately after the TST, mice were subjected to OFT (Steru *et al.*, 1985).

Open field test (OFT)

The possible influence of the treatments on the locomotor activity of mice was assessed using the OFT, as previously described (Dalmagro, Camargo, Zeni, 2017). The apparatus consisted of a wooden box (40 × 60 × 50 cm) with the floor divided into 12 equal squares. An experienced observer, blinded to the experimental groups,

recorded the number of squares crossed by mice with all four paws (crossings) for 6 min. During the experiment, minimum light was ensured to avoid anxiogenic behavior. The arena was cleaned between tests with 10% ethanol.

Statistical analysis

Data were analyzed using GraphPad Prism 7.0 (GraphPad Software, Inc., San Diego, CA). Results of the anti-immobility effect of caffeic acid were evaluated by one-way analysis of variance (ANOVA) followed by Dunnett's test, whereas the results regarding the involvement of the cellular signaling pathways were analyzed by two-way ANOVA followed by Tukey's test. The differences between groups were considered significant when $p < 0.05$.

RESULTS AND DISCUSSION

Antidepressant-like effect of caffeic acid acutely administered to mice

Caffeic acid, a phenolic compound present in food, coffee, wine, and plants, has been studied for its

antidepressant-like and anxiolytic-like effects in mice (Hall *et al.*, 2015). The efficacy of caffeic acid in different models of stress indicates that it can inhibit emotional changes caused by stress, such as anxiety and depression. Takeda *et al.* (2003) also suggested that caffeic acid can indirectly modulate $\alpha 1$ -adrenoceptor. Another important activity of caffeic acid is the inhibition of 5-lipoxygenase enzyme, which is involved in inflammation and arachidonic acid metabolism. In addition, it triggers neuroprotective effects in neuronal cells, as evaluated by an *in vitro* study (Cai *et al.*, 2016; Taram, Winter, Linseman, 2016).

In the present study, we used the TST to assess the antidepressant-like effect of caffeic acid in mice. Moreover, the OFT was used to investigate the possible influence of treatments on mice locomotor activity. Mice were treated 1 h before conducting the behavioral tests with the vehicle, caffeic acid (5, 10, 15 mg/kg, p.o.) and fluoxetine (20 mg/kg, p.o.).

All doses of caffeic acid reduced the immobility time of mice, as assessed using TST ($p < 0.0001$; $p < 0.001$; $p < 0.01$, respectively - Figure 1 A). No changes were observed regarding locomotion ($p > 0.05$; Figure 1 B) of mice. Based on these preliminary data, caffeic acid at a dose of 5 mg/kg was selected for subsequent experiments.

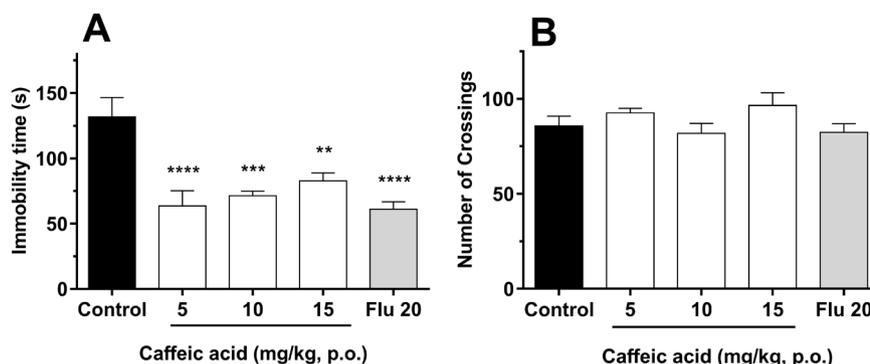


FIGURE 1 - Caffeic acid antidepressant-like effect in mice subjected to TST. Mice were treated with caffeic acid (5, 10, 15 mg/kg, p.o.) or fluoxetine (20 mg/kg, p.o.) 1h before the TST and OFT. Each column represents the mean \pm S.E.M. ($n=8-10$). ** $p < 0.01$; *** $p < 0.001$ and **** $p < 0.0001$ as compared with the control group. Results were analyzed by one-way ANOVA followed by Dunnett's test.

Involvement of the cellular signaling pathways in the acute antidepressant-like effect of caffeic acid

Several studies have suggested that depressive disorders are related to neuronal death in specific regions, dysfunctions in the intracellular signaling pathways, and neuroplasticity changes. Studies assessing depression and alterations in the signaling pathways that regulate neuroplasticity and cell survival have been extensively reported (Ampuero *et al.*, 2010; Castrén, Kojima, 2017). These signaling pathways and signal transductions are essential for the appropriate functioning of the central nervous system, and represent strategic targets for the development of potential therapeutic agents for mood disorders (Castrén, Hen, 2013). In this context, this study investigated the involvement of the cell signaling pathways mediated by PKA, MAPK/ERK, and PKC in the antidepressant-like effect of caffeic acid in mice subjected to TST.

PKA and PKC mediate their functions by modulating the phosphorylation of specific substrates, such as CREB,

which when activated, induces the expression of target genes and neurotrophins, such as BDNF, promoting cell survival, neuronal plasticity, and mood modulation. The MAPK/ERK pathway is involved in the regulation of numerous cellular processes, such as cell proliferation and survival, as well as CREB phosphorylation. In our study, among the family of MAPKs, ERK1 and ERK2 were studied to identify their involvement in the mechanism underlying antidepressant activity. When BDNF binds to its receptor (TrkB-tropomyosin-related kinases B), the MAPK cascade is activated, in addition to the activation of ERK1 and 2 (Ampuero *et al.*, 2010; Castrén, Hen, 2013; 2017; Niciu *et al.*, 2013). This event induces the phosphorylation of nuclear gene transcription factors (Pandey *et al.*, 2004).

Figure 2 shows the effect of inhibitor pretreatment on the anti-immobility effect of caffeic acid (5 mg/kg, p.o.) in mice. As assessed using the TST, mice administered with H-89 (a PKA inhibitor) plus caffeic acid demonstrated an increase in the immobility time (Figure 2 – A, $p < 0.01$) compared with mice treated only with the phenolic acid.

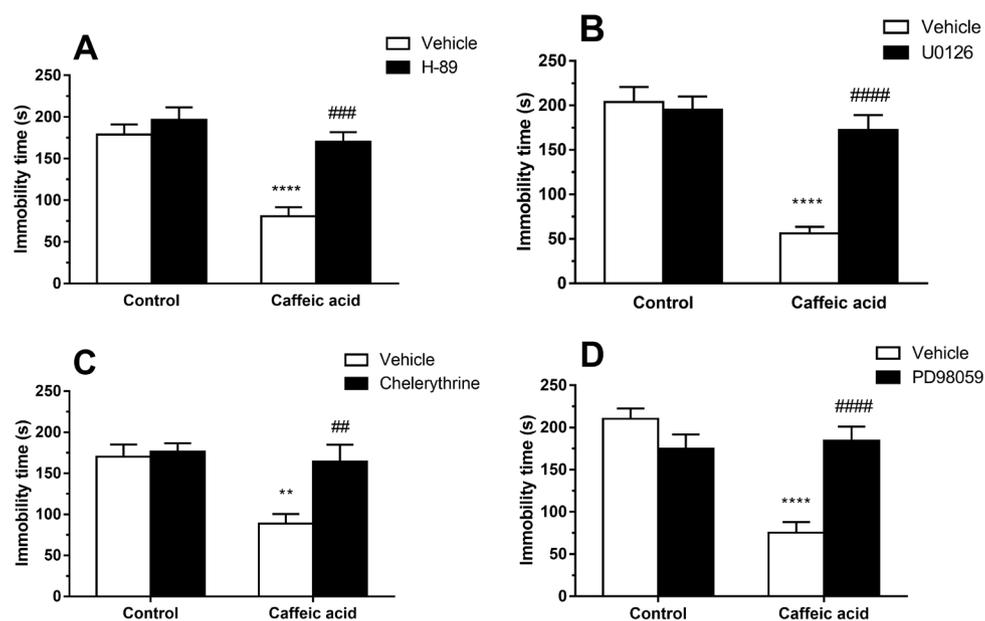


FIGURE 2 - Involvement of the cellular signaling pathways in the acute antidepressant-like effect of caffeic acid in mice. Effect of pretreatment of mice with H-89 (1 μ g/site, i.c.v.) (A), U0126 (5 μ g/site, i.c.v.) (B), chelerythrine (1 μ g/site, i.c.v.) (C) and PD98059 (5 μ g/site, i.c.v.) (D) and, 30 min later, they received caffeic acid (5 mg/kg, p.o.) or vehicle (p.o.). TST was conducted 1h after the administration of caffeic acid or vehicle. Each column represents the mean \pm S.E.M. $n=8-10$ animals; ** $p < 0.01$; *** $p < 0.001$, and **** $p < 0.0001$ as compared to the control group (vehicle). ## $p < 0.01$; #### $p < 0.001$, and ##### $p < 0.0001$ as compared to the same group pretreated with vehicle. Results were analyzed by two-way ANOVA followed by Tukey's test.

Pre-administration of U0126 (MEK 1/2 inhibitor) to mice increased the immobility time of animals that also received caffeic acid (Figure 2 – B, $p < 0.0001$), indicating an influence of this pathway in the antidepressant-like effect of caffeic acid. The involvement of PKC in the pharmacological effect of caffeic acid was also assessed by the pre-administration of chelerythrine in mice, a PKC inhibitor. The treatment of animals with caffeic acid and chelerythrine increased their immobility time as evaluated using the TST (Figure 2 – C, $p < 0.01$). Moreover, the MAPK/ERK pathway was involved in the antidepressant-like effect of caffeic acid as PD98059 pre-administration (MAPK-ERK inhibitor) reversed the anti-immobility effect of phenolic acid in mice (Figure 2 – D, $p < 0.0001$). Briefly, our results suggest that PKA, PKC, MAPKs, and MEK 1/2 cellular signaling pathways simultaneously mediate the antidepressant-like

effect of caffeic acid. However, several investigations must be conducted to clarify these issues; however, the antidepressant-like effect of caffeic acid seems to be linked to α_1 -adrenoreceptor modulation (Takeda *et al.*, 2003). Previous reports demonstrated that multiple signaling pathways are activated in response to α_1 -adrenoreceptor modulation (cAMP/PKA, PKC, ERK, PI3K), which explains, at least partially, our results (Copik *et al.*, 2015; Jiang *et al.*, 2009; Scarparo, Visconti, Castrucci, 2006; Segura *et al.*, 2013).

We also evaluated the possible interferences elicited by treatments in the locomotor activity of mice through OFT. The administration of vehicle, inhibitors, caffeic acid, and the association between inhibitors plus caffeic acid, did not affect mice's locomotor activity, as shown in Figure 3 A, B, C, and D ($p > 0.05$).

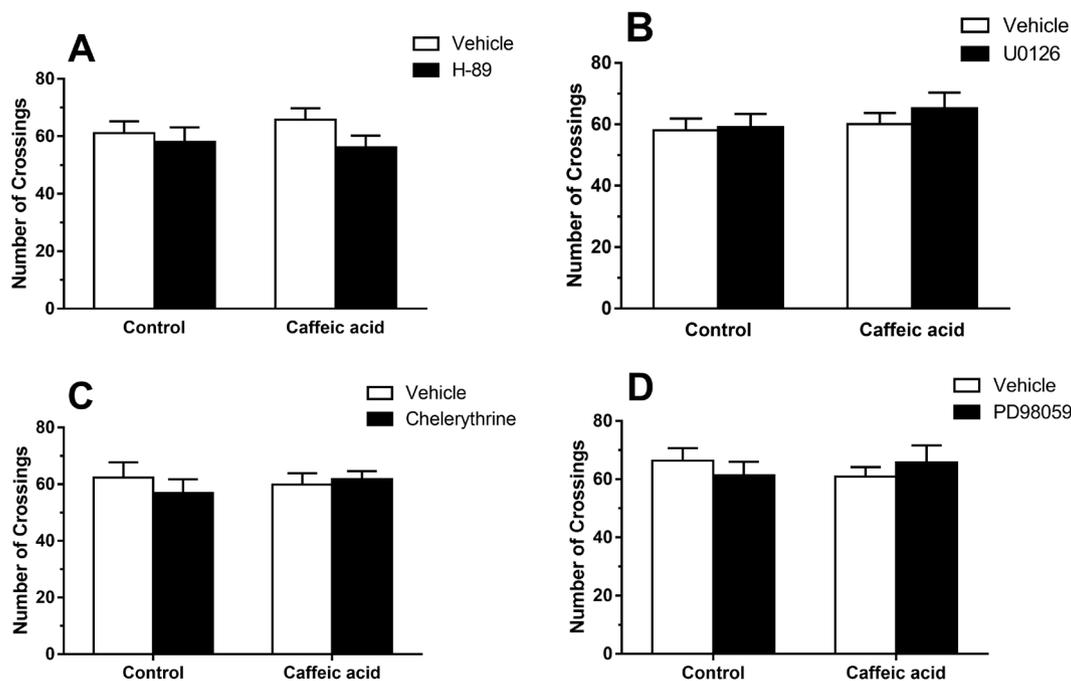


FIGURE 3 - Influence of the treatments in the locomotor activity of mice evaluated in the OFT. Effect of pretreatment of mice with H-89 (1 $\mu\text{g}/\text{site}$, i.c.v.) (A), U0126 (5 $\mu\text{g}/\text{site}$, i.c.v.) (B), chelerythrine (1 $\mu\text{g}/\text{site}$, i.c.v.) (C) and PD98059 (5 $\mu\text{g}/\text{site}$, i.c.v.) (D) and, 30 min later, they received caffeic acid (5 mg/kg, p.o.) or vehicle (p.o.). TST and OFT were conducted 1h after the administration of caffeic acid or vehicle. Each column represents the mean \pm S.E.M. $n=8-10$ animals – $p > 0.05$. Results were analyzed by two-way ANOVA followed by Tukey's test.

In summary, we investigated the possible cell signaling pathways involved in the antidepressant-like effect of caffeic acid. The behavioral changes induced by phenolic acid could be mediated by PKA, MEK 1/2, PKC, and MAPKs, reinforcing the hypothesis that this compound is promising for the pharmacological treatment of mood disorders.

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DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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