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Role of potassium channels in the antidepressant-like effect of folic acid in the forced swimming test in mice

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ABSTRACT

Potassium (K^+) channels have been implicated in depressive disorders and in the mechanism of action of antidepressants. Considering that several studies have indicated that folic acid plays an important role in the pathophysiology of depression, the present study investigated the involvement of potassium channels in the antidepressant-like effect of this vitamin. For this aim, the effect of the combined administration of different types of K^+ channel blockers and folic acid in the forced swimming test (FST) was investigated. Treatment of mice by intracerebroventricular (i.c.v.) route with subactive doses of glibenclamide (an ATP-sensitive K^+ channels blocker, 0.5 pg/site), charybdotoxin (a large- and intermediate-conductance calcium-activated K^+ channel blocker, 25 pg/site) or apamin (a small-conductance calcium-activated K^+ channel blocker, 10 pg/site), augmented the effect of folic acid (10 mg/kg, p.o., subeffective dose) in the FST. Additionally, the administration of folic acid and the K^+ channel blockers, alone or in combination, did not affect locomotion in the open-field test. Moreover, the reduction in the immobility time in the FST elicited by folic acid administered at a higher dose (50 mg/kg, p.o.) was prevented by the pretreatment of mice with the K^+ channel opener cromakalim (10 μg/site, i.c.v.), without affecting locomotor activity. The results of this study indicate that the antidepressant-like effect of folic acid in the FST may be at least partly due to its modulatory effects on neuronal excitability, via inhibition of K^+ channels.

1. Introduction

Depression is a chronic, severe and debilitating mental illness that affects millions of people worldwide. Although the underlying pathophysiological mechanisms of depression are not completely established, novel targets have been identified for the development of new pharmacological treatments (Lee et al., 2010). There is increasing evidence that a folic acid status is an important factor that may contribute to depressive disorders and its treatment (Coppen and Bolander-Gouaille, 2005; Morris et al., 2008; Sarris et al., 2009). Folic acid (folate), one of the 13 essential vitamins which is obtained from dietary sources or supplements, is essential for the functioning of nervous system, since it displays an important role in neuroplasticity and maintenance of neuronal integrity (Fenech, 2010; Kronenberg et al., 2009).

Many important metabolic processes are dependent on folic acid availability, including the synthesis of norepinephrine, dopamine and serotonin, which are neurotransmitters implicated in the pathogenesis and treatment of depression (Fava and Mischoulon, 2009). There are several clinical studies regarding folic acid deficiency associated with a higher incidence of depression. These studies show that: a) reduced plasma, serum or red blood cell folic acid is commonly found in major depressive illnesses (Abou-Saleh and Coppen, 2006; Sarris et al., 2009); b) a low folic acid status is associated with poorer response to antidepressant medication; on the other hand, folic acid supplementation added to antidepressant medication improves its therapeutic effect (Alpert et al., 2002; Coppen and Bailey, 2000; Godfrey et al., 1990). Low folic acid status is associated with reduced serotonergic and/or neurotransmitter function; (c) preclinical studies from our group have shown that systemic and central administration of folic acid produces antidepressant-like effect in two predictive models of antidepressant activity, the forced swimming test (FST) and tail suspension test (TST) (Brocardo et al., 2008a). The mechanisms by which folic acid produces antidepressant-like effect are not fully established, but they were shown to be dependent on the serotonergic and noradrenergic systems (Brocardo et al., 2008a), inhibition of N-methyl-D-aspartic acid (NMDA) receptors and nitric oxide (NO) and cyclic guanosine monophosphate (cGMP) synthesis (Brocardo et al., 2008b). Additionally, antidepressant-like effect of folic acid is also mediated by an interaction with the opioid system (μ_1- and δ-opioid receptors) (Brocardo et al., 2009), inhibition of glycogen synthase kinase-3 (GSK-3β) and activation of peroxisome proliferator-activated receptor-γ (PPARγ) (Budni et al., 2011b).

NO is an important messenger in the central nervous system. It is produced from L-arginine by the catalytic action of NO synthase

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(NOS). Physiologically, NO actions may be mediated by locally produced NO and in most instances by the subsequently generated second messenger molecule guanosine 3′5 cyclic monophosphate. Studies indicate that different types of K^+ channels in several tissues can be activated by NO *per se* or through cGMP production (Jeong et al., 2001; Shin et al., 1997). Additionally, a previous study of our group demonstrated that the antidepressant-like effect elicited by the inhibition of several subtypes of K^+ channels is dependent on the inhibition of NO–cGMP synthesis (Kaster et al., 2005). Thus, K^+ channels might be one of the physiological targets of NO in the brain (Jeong et al., 2001) and the inhibition of these channels might play an important role in the pathophysiology of depression.

Therefore, the aim of this study was to investigate whether the blockade of K^+ channels can contribute to the antidepressant-like effect of folic acid in the FST in mice.

2. Materials and methods

2.1. Animals

Adult Swiss mice of either sex (homogeneously distributed among groups), weighing 30–40 g were maintained at 20–22 °C with free access to water and food, under a 12:12 h light:dark cycle (lights on at 7:00 am). Male and female mice were maintained in different cages. All manipulations were carried out between 9:00 am and 4:00 pm, with each animal used only once. All procedures were performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals. The experiments were performed after approval by the Ethics Committee of the Institution and all efforts were made to minimize animal suffering and to reduce the number of animals used in the experiments.

2.2. Drugs and treatment

The following drugs were used: folic acid (Sigma Chemical Co., St. Louis, U.S.A.), charybdotoxin, cromakalim and glibenclamide (Tocris Cookson, Ballwin, MO, USA). Cromakalim was dissolved in saline with 10% Tween 80, whereas all the other drugs were dissolved in isotonic saline solution (NaCl 0.9%) immediately before use, except folic acid which was dissolved in distilled water. Appropriate vehicle-treated groups were also assessed simultaneously. All the drugs were administered by intracerebroventricular (i.c.v.) route, in a volume of 5 μl per mouse, except folic acid which was administered by oral route (p.o.) in a constant volume of 10 ml/kg body weight. I.c.v. injections were given under light ether anesthesia, directly into the lateral ventricle as described previously by Budni et al. (2007), with the bregma fissure as a reference. Vehicle, potassium channel blockers or potassium channel opener were injected in a volume of 5 μl, given over 30 s, and the cannula remained in place for another 30 s.

To test the hypothesis that the antidepressant-like effect of folic acid is mediated through the inhibition of K^+ channels, animals were pretreated with a subeffective dose of folic acid (10 mg/kg, p.o.), and 45 min later they received subeffective doses of glibenclamide (an ATP-sensitive K^+ channel blocker, 0.5 pg/site), charybdotoxin (a large- and intermediate-conductance calcium-activated K^+ channel blocker, 25 pg/site) or apamin (a small-conductance calcium-activated K^+ channel blocker, 10 pg/site) before being tested in the FST.

In order to rule out any psychostimulant effect of the interaction of K^+ channel blockers and folic acid, mice were pretreated by oral route with folic acid 45 min before the administration by i.c.v. route of glibenclamide (0.5 pg/site), charybdotoxin (25 pg/site) or apamin (10 pg/site). The open-field test was carried out 15 min later.

In another set of experiments, mice were pretreated with folic acid (50 mg/kg, p.o.), 45 min before the administration of cromakalim (a K^+ channel opener, 10 μg/site, i.c.v.). FST or the open-field test was carried out 15 min later.

The doses of folic acid were chosen based on previous studies from our group (Brocardo et al., 2008a, 2008b, 2009; Budni et al., 2011b).

The doses of glibenclamide, charybdotoxin, apamin and cromakalim were chosen on the basis of literature and are previously reported not to increase locomotor activity (Budni et al., 2007; Galeotti et al., 1999; Kaster et al., 2005, 2007).

2.3. Forced swimming test (FST)

Briefly, mice were individually forced to swim in an open cylindrical container (diameter 10 cm, height 25 cm), containing 19 cm of water (depth) at 25 ± 1 °C; the total duration of immobility was measured during 6-min period as described previously (Brocardo et al., 2008a; Budni et al., 2007; Kaster et al., 2005). Each mouse was judged to be immobile when it ceased struggling and remained floating motionless in the water, making only those movements necessary to keep its head above water. A decrease in the duration of immobility is indicative of an antidepressant-like effect (Porsolt et al., 1977).

2.4. Open-field test

To assess the possible effects of folic acid on locomotor activity, mice were evaluated in the open-field paradigm as previously described (Budni et al., 2007; Rodrigues et al., 1996). Animals were individually placed in a wooden box (40 × 60 × 50 cm) with the floor divided into 12 rectangles. The number of squares crossed with all paws (crossing) was counted in a 6 min session. The apparatus were cleaned with a solution of 10% ethanol between tests in order to hide animal clues.

2.5. Statistical analysis

All experimental results are given as the mean ± SEM. Comparisons between experimental and control groups were performed by two-way ANOVA (interaction of folic acid with the pharmacological agents) followed by Newman–Keuls test when appropriate. A value of $p<0.05$ was considered to be significant.

3. Results

3.1. Effects of combined administration of subeffective doses of the K^+ channel blockers and folic acid in the FST

The results presented in Fig. 1A show the synergistic antidepressant-like effect of glibenclamide (an ATP-sensitive K^+ channel blocker, 0.5 pg/site, i.c.v.) combined with a subeffective dose of folic acid (10 mg/kg, p.o.) in the FST. The two-way ANOVA revealed a significant effect of glibenclamide treatment [$F_{1,28}=12.78$, $p<0.01$] and pretreatment × treatment interaction [$F_{1,28}=16.30$, $p<0.01$], but not of folic acid pretreatment [$F_{1,28}=2.49$, $p=0.12$].

Fig. 1B shows that apamin (a small-conductance calcium-activated K^+ channel blocker, 10 pg/site, i.c.v.) in combination with a subeffective dose of folic acid reduced the immobility time in the FST when compared with either drug alone. The two-way ANOVA revealed a significant effect of apamin treatment [$F_{1,28}=14.69$, $p<0.01$] and pretreatment × treatment interaction [$F_{1,28}=11.28$, $p<0.01$], but not of folic acid pretreatment [$F_{1,28}=2.67$, $p=0.11$].

As presented in Fig. 1C, the administration of charybdotoxin (a large- and intermediate-conductance calcium-activated K^+ channel blocker, 25 pg/site, i.c.v.) in combination with subeffective dose of folic acid also produced an antidepressant-like effect in the FST. The two-way ANOVA revealed a significant effect of folic acid pretreatment [$F_{1,28}=8.75$, $p<0.01$], charybdotoxin treatment [$F_{1,28}=6.72$,