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Antidepressant-like effect of folic acid: Involvement of NMDA receptors and L-arginine-nitric oxide-cyclic guanosine monophosphate pathway

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ABSTRACT

Antidepressant-like activity of folic acid in forced swimming test and in the tail suspension test was demonstrated previously by our group. In this study we investigated the involvement of *N*-methyl-D-aspartate (NMDA) receptors and L-arginine-nitric oxide (NO)-cyclic guanosine monophosphate pathway in its antidepressant-like effect in the forced swimming test in mice. The antidepressant-like effect of folic acid (10 nmol/site, i.c.v.) was prevented by the pretreatment of mice with NMDA (0.1 pmol/site, i.c.v.), L-arginine (750 mg/kg, i.p., substrate for nitric oxide synthase), *S*-nitroso-*N*-acetyl-penicillamine (SNAP, 25 µg/site, i.c.v, a NO donor) or sildenafil (5 mg/kg, i.p., phosphodiesterase 5 inhibitor). The administration of 7-nitroindazole (25 and 50 mg/kg, i.p., a specific neuronal nitric oxide synthase (nNOS) inhibitor) or methylene blue (20 mg/kg, i.p., direct inhibitor of both nitric oxide synthase and soluble guanylate cyclase) in combination with a sub-effective dose of folic acid (1 nmol/site, i.c.v.) reduced the immobility time in the FST as compared with either drug alone. Together the results suggest that the antidepressant-like effect of folic acid in the forced swimming test is dependent on an inhibition of either NMDA receptors or NO and cGMP synthesis.

1. Introduction

Depressive disorders represent a major public health problem due to their high prevalence and psychosocial impact. The WHO ranks unipolar depression as the fourth most important cause of mortality and disability (Murray and Lopez, 1997). Although the underlying pathophysiological mechanisms of depression are not completely identified, novel targets have been identified for the development of new pharmacological and behavioral treatments. Recently, there is increasing evidence that a disturbed one-carbon metabolism may be a significant factor contributing to depressive disorders (Coppen and Bolander-Gouaille, 2005).

Folic acid (folate) is a water-soluble vitamin that is essential for cell replication. Folate is a major determinant of one-carbon metabolism, in which *S*-adenosylmethionine is formed, that, in turn, donates methyl groups that are crucial for neurological function. Increased plasma homocysteine is a functional marker of folate deficiency, and increased homocysteine levels are found in depressive patients (Bottiglieri, 2005). Reduced plasma, serum, or red blood cell folate is commonly found in major depressive illnesses (Bottiglieri, 2005; Abou-Saleh and Coppen, 2006). Moreover, supplementing antidepressant medication with folic acid enhances the therapeutic effect (Godfrey et al., 1990; Coppen and Bailey, 2000; Alpert et al., 2002).

Pre-clinical and clinical data have suggested that NMDA receptor antagonists and compounds that reduce transmission at NMDA receptors exhibit antidepressant properties (Skolnick, 1999; Paul and Skolnick, 2003; Zarate et al., 2006). The NMDA receptor stimulation induces the activation of nitric oxide (NO) synthase (NOS). The activated NOS then converts L-arginine to NO and L-citrulline (Esplugues, 2002). NO, induced by NMDA receptor stimulation has recently been implicated in the regulation of various behavioral, cognitive and emotional processes, including depression (Harkin et al., 1999; Da Silva et al., 2000; Wegener et al., 2003). The relationship between the production of NO and the NMDA receptor complex as well as the antidepressant-like effects of functional NMDA antagonists have led to studies of the putative antidepressant action of NOS inhibitors. It has been demonstrated that NOS inhibitors exert antidepressant-like effects in the forced swimming test (Harkin et al., 1999; Da Silva et al., 2000; Karolewicz et al., 2001; Volke et al., 2003). Moreover, the administration of NOS inhibitors was also reported to cause an increase in the effects of 5-HT reuptake inhibitors in the forced swimming test (Harkin et al., 2004).

Further support for the hypothesis that the inhibition of NO synthesis, with a subsequent decrease in the concentration of cGMP (Snyder, 1992), may produce antidepressant-like effects, at least under certain conditions, comes from the reported reduction in the immobility time in the forced swimming test elicited either by the administration of methylene blue, which acts as a direct inhibitor of both NOS and soluble guanylate cyclase (sGC) (Eroglu and Caglayan, 1997) or by the specific inhibitor of soluble guanylate cyclase activity,

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1H-(1,2,4)-oxodiazolo (4,3-a)quinoxalin-1-one, ODQ (Heiberg et al., 2002; Kaster et al., 2005; Ergün and Ergün, 2007).

Although the antidepressant-like effect of folic acid in the mouse forced swimming test has been reported (Brocardo et al., 2008) little is known concerning its mechanism of action. Due to the existence of data indicating that the NO-cGMP pathway is involved in the pathophysiology of depression (Harkin et al., 1999; Yildiz et al., 2000; Heiberg et al., 2002; Volke et al., 2003) this study investigated the participation of the L-arginine-NO-cGMP pathway in the antidepressant-like effect of an acute administration of folic acid in the forced swimming test.

2. Materials and methods

2.1. Animals

Swiss mice of either sex, weighing 30–40 g were maintained at 22–25 °C with free access to water and food, under a 12:12 h light:dark cycle (lights on at 7:00 h). All manipulations were carried out between 9:00 and 16:00 h, with each animal used only once. The experiments were performed after approval of the protocol by the Ethics Committee of the Institution and all efforts were made to minimize animal suffering.

2.2. Drugs and treatment

The following drugs were used: folic acid, L-arginine, methylene blue, NMDA, *S*-nitroso-*N*-acetyl-penicillamine (SNAP), sildenafil and 7-nitroindazole (Sigma Chemical Co, USA). All drugs were dissolved in saline except 7-nitroindazole that was dissolved in few drops of Tween 80. Most of the drugs were administered by intraperitoneal (i.p.) route in a constant volume of 10 ml/kg body weight, except folic acid, SNAP and NMDA which were administered by intracerebroventricular (i.c.v.) route (5 µl/site).

To test the hypothesis that the antidepressant-like effect of folic acid is mediated through the inhibition of NMDA receptors, mice (n=6) were pretreated with NMDA (0.1 pmol/site, i.c.v.) and 15 min after, folic acid (10 nmol/site) or vehicle was administered. Fifteen minutes later the forced swimming test was carried out. The dose of NMDA was chosen based on a dose–response curve carried out by our group in which NMDA caused neither overt signs of toxicity nor alteration in the locomotor activity. Higher NMDA doses (1–1000 pmol/site, i.c.v.) caused convulsion.

To investigate whether the antidepressant-like effect of folic acid is mediated through the involvement of the L-arginine-nitric oxide pathway in the forced swimming test, mice (n=6) were pretreated with L-arginine, a precursor of nitric oxide (750 mg/kg, i.p., a dose that produces no effect in the forced swimming test) or with the NO donor SNAP (25 µg/site, i.c.v., a dose that produces no effect in the forced swimming test). Thirty minutes after L-arginine or 15 min after SNAP administration, folic acid (10 nmol/site) or vehicle was injected, and 15 min later the forced swimming test was carried out. The dose of L-arginine was chosen based on a previously reported dose–response curve in the forced swimming test (Da Silva et al., 2000). The dose of SNAP was chosen based on a dose–response curve carried out by our group (data not shown).

In another experiment, we also investigated the effect of folic acid (1 nmol/site, a sub-effective dose) with sub-effective doses of 7-nitroindazole (25 and 50 mg/kg, i.p., a specific neuronal NO synthase inhibitor) or methylene blue (10–20 mg/kg, i.p., an inhibitor of both NO synthase and soluble guanylate cyclase). Folic acid or vehicle was administered 20 min before the drugs and 15 min later the animals (n=5–6) were tested in the forced swimming test.

To investigate the role of cyclic GMP (cGMP) in the antidepressant action of folic acid, mice (n=6) received an injection of sildenafil (5 mg/kg, i.p., a phosphodiesterase (PDE) inhibitor), or vehicle 30 min before folic acid (10 nmol/site); a further 15 min elapsed before the animals were tested in the forced swimming test. The dose of sildenafil was chosen based on the work from Kaster et al. (2005). This dose was reported to reverse the antidepressant-like effect of several compounds (Almeida et al., 2006; Dhir and Kulkarni, 2007; Kulkarni and Dhir, 2007).

2.3. Intracerebroventricular injection

Intracerebroventricular (i.c.v.) administration was performed under ether anesthesia. Briefly, a 0.4 mm external diameter hypodermic needle attached to a cannula, which was linked to a 25 µl Hamilton syringe, was inserted perpendicularly through the skull and no more than 2 mm into the brain of the mouse. A volume of 5 µl was then administered in the left lateral ventricle. The injection was given over 30 s, and the needle remained in place for another 30 s in order to avoid the reflux of the substances injected. 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. To ascertain that the drugs were administered exactly into the cerebral ventricle, the brains were dissected and examined macroscopically after the test.

2.4. Forced swimming test

Mice were individually forced to swim in an open cylindrical container (diameter 10 cm, height 25 cm), containing 19 cm of water at 25±1 °C; the total duration of immobility during a 6 min test was scored as described previously (Rosa et al., 2003; 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.

2.5. Open-field test

The forced swimming test has some drawbacks represented by the possibility of obtaining false positives or negatives. Drugs enhancing

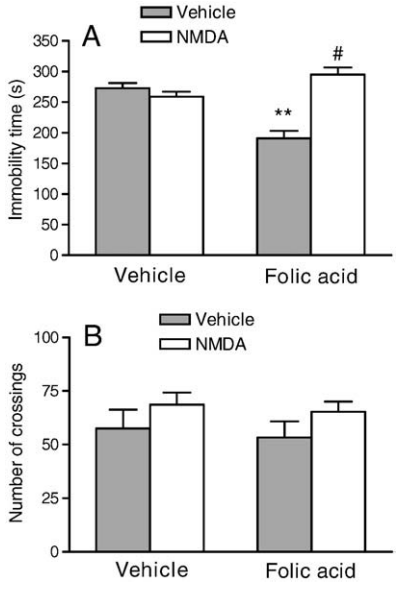

Fig. 1. Effect of the pretreatment of mice with NMDA (0.1 pmol/site, i.c.v.) on the anti-immobility action of folic acid (10 nmol/site, i.c.v.) in the forced swimming test (panel A) and on the number of crossings in the open-field test (panel B). Values are expressed as mean + S.E.M. (n=6–7). **P<0.01 compared with the vehicle-treated control; #P<0.01 compared with the same group pretreated with vehicle. A) pretreatment [F(1,22)=18.04, P<0.01]; treatment [F(1,22)=4.57, P<0.05]; pretreatment×treatment interaction [F(1,22)=30.60, P<0.01]. B) pretreatment [F(1,20)=3.82, P=0.06]; treatment [F(1,20)=0.35, P=0.55]; pretreatment×treatment interaction [F(1,20)=0.0005, P=0.98].