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Involvement of L-arginine–nitric oxide–cyclic guanosine monophosphate pathway in the antidepressant-like effect of venlafaxine in mice

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Abstract

The involvement of L-arginine–nitric oxide (NO)–cyclic guanosine monophosphate (cGMP) signaling pathway in the antidepressant action of venlafaxine (dual serotonin and norepinephrine reuptake inhibitor) was investigated in mice. The antidepressant activity was assessed in forced swim test (FST) behavioral paradigm. Total immobility time was registered during the period of 6 min. Venlafaxine produced dose-dependent (4–16 mg/kg, i.p.) reduction in immobility period. The antidepressant-like effect of venlafaxine (8 mg/kg, i.p.) was prevented by pretreatment with L-arginine (750 mg/kg, i.p.) [substrate for nitric oxide synthase (NOS)]. Pretreatment of mice with 7-nitroindazole (7-NI) (25 mg/kg, i.p.) [a specific neuronal nitric oxide synthase (nNOS) inhibitor] produced potentiation of the action of subeffective dose of venlafaxine (2 mg/kg, i.p.). In addition, treatment of mice with methylene blue (10 mg/kg, i.p.) [direct inhibitor of both nitric oxide synthase (NOS) and soluble guanylate cyclase (sGC)] potentiated the effect of venlafaxine (2 mg/kg, i.p.) in the FST. Furthermore, the reduction in the immobility time elicited by venlafaxine (8 mg/kg, i.p.) was also inhibited by pretreatment with sildenafil (5 mg/kg, i.p.) [phosphodiesterase 5 inhibitor]. The various modulators used in the study did not produce any changes in locomotor activity *per se*. The results demonstrated that the antidepressant-like effect of venlafaxine in the FST involved an interaction with the L-arginine–NO–cGMP pathway.

Keywords: Cyclic GMP; Forced swim test (FST); L-arginine; Nitric oxide; Phosphodiesterase 5 inhibitor; Venlafaxine

1. Introduction

Venlafaxine, a novel antidepressant is an inhibitor of both serotonin and norepinephrine transporters (SERT and NET, respectively) (Redrobe et al., 1998). It exhibits six- to seven-fold selectivity for inhibition of serotonin reuptake as compared to norepinephrine reuptake in synaptosome of rat brain and a 15- to 30-fold higher affinity for SERT binding sites as compared to those of NET (Gould et al., 2006). Venlafaxine has been shown to be superior in efficacy to selective serotonin reuptake inhibitors (SSRIs) in severe major depressive disorder, treatment-resistant depression, depressive symptom remission and obsessive compulsive disorder (Gutierrez et al., 2003).

L-arginine–nitric oxide (NO)–cyclic guanosine monophosphate (cGMP) is an important signaling pathway that is reported to be involved in depression (Mantovani et al., 2003). Nitric oxide, a messenger molecule in the brain, is synthesized from L-arginine by nitric oxide synthase (NOS), and has been implicated in neurotransmission, synaptic plasticity, learning, perception of pain, aggression and depression (Esplugues, 2002). Recent evidence have shown that the reduction of NO levels within the hippocampus can induce antidepressant-like effects, thus implicating endogenous hippocampal NO in the neurobiology of stress and depression (Joca and Guimaraes, 2006). Several of the physiological actions of NO are mediated through its interaction with the heme iron of soluble guanylate cyclase (sGC), leading to enzyme activation and consequent increase in guanosine cyclic monophosphate (cGMP) (Kaster et al., 2005a). Recent studies have shown the possibility that the inhibition of NO synthase could be used as a strategy to enhance the clinical efficacy of serotonergic antidepressants (Harkin et al., 2004). The present study attempts to investigate the

Abbreviations: AMP, Adenosine monophosphate; cGMP, Cyclic guanosine monophosphate; FST, Forced swim test; NET, Norepinephrine transporter; NO, Nitric oxide; NOS, Nitric oxide synthase; PDE, Phosphodiesterase; SERT, Serotonin transporter; sGC, soluble guanylate cyclase.

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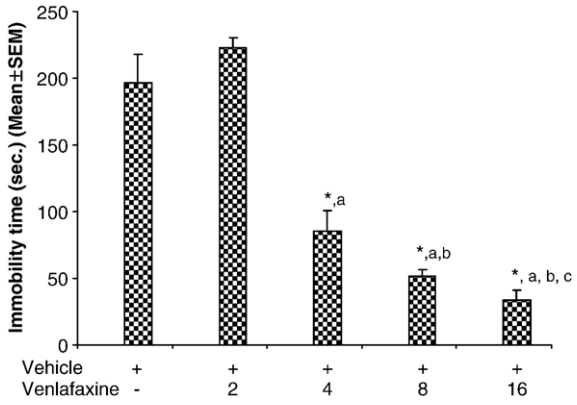

Fig. 1. Effect of different doses of venlafaxine (2, 4, 8 and 16 mg/kg) on the mean immobility period in mouse FST. The values are expressed as mean±S.E.M. (n=6–8). Data was analysed by One-Way Analysis of Variance (ANOVA) followed by Dunnett's test. *P<0.001 compared with the vehicle-treated control. [a]P<0.01 compared with the venlafaxine (2 mg/kg, i.p.) group. [b]P<0.01 compared with the venlafaxine (4 mg/kg, i.p.) group. [c]P<0.01 compared with the venlafaxine (8 mg/kg, i.p.) group.

participation of L-arginine–NO–cGMP pathway in the antidepressant activity of venlafaxine in FST in mice.

2. Materials and methods

2.1. Animals

Male albino mice (Laca strain) weighing between 22 and 30 g bred in the Central Animal House (CAH) facility of the Panjab University, Chandigarh, India were used. The animals were housed under standard laboratory conditions and maintained on natural light and dark cycle, and had free access to food and water. Animals were acclimatized to laboratory conditions before the experiment. Each animal was used only once. All the experiments were carried out between 0900 and 1500 h. The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) and conducted according to the Indian National Science Academy (INSA) Guidelines for the use and care of experimental animals.

Table 1
Effect of different doses of venlafaxine (2–16 mg/kg, i.p.) *per se* and its combination with L-arginine, 7-NI, methylene blue or sildenafil on the locomotor activity measured for a total of 5-minute session

Serial no.	Treatment	Dose (mg/kg, i.p.)	Mean ambulatory movement
1	Vehicle control		200±8.72
2	Venlafaxine	2	196±6.24
		4	212±6.95
		8	227±6.21
		16	284±3.25 *
3	L-arginine	750	187±9.65
4	L-arginine+venlafaxine	750+8	216±5.02
5	7-nitroindazole	25	215±6.58
6	7-nitroindazole+venlafaxine	25+2	207±6.36
7	Methylene blue	10	198±3.65
8	Methylene blue+venlafaxine	10+2	190±4.27
9	Sildenafil	5	186±9.65
10	7-NI+sildenafil	5+8	185±8.65

The values are expressed as mean±S.E.M. (n=6–8).
* P<0.01 compared to control group.

2.2. Experimental procedure

2.2.1. Forced swim test (FST)

The test procedure was carried out according to the previously standardized and validated animal model in our laboratory (Kulkarni and Mehta, 1985; Parale and Kulkarni, 1986; Reddy et al., 1998). In brief, mice were individually forced to swim inside a rectangular glass jar (25×12×25 cm^3 containing 15 cm of water maintained at 23–25 °C). After the initial 2–3 min of vigorous activity the animals showed period of immobility by floating with minimum movements. An animal is considered to be immobile whenever it remained floating passively in the water in a slightly hunched but upright position, its nose above the water surface. The total immobility time for the period of 6 min was recorded with the help of a stop-watch (Kulkarni and Mehta, 1985).

2.2.2. Measurement of locomotor activity

Locomotor activity (ambulations) was measured by using a computerized actophotometer (IMCORP, India). An array of 16 infrared emitter, detector pairs measured animal activity along a single axis of motion, the digital data being displayed on the front panel meters as ambulatory movements. Mice were allowed to acclimatize to the observation chamber for a period of 2 min. Locomotion was expressed in terms of total photobeams counts per 5 min per animal (Dhir et al., 2005).

2.3. Drugs and treatment

The following drugs were used: venlafaxine (Panacea Biotec Ltd, New Delhi, India), L-arginine (Loba-Chemie, Mumbai, India), methylene blue (S.D.-fine Chem Ltd., Gujarat,

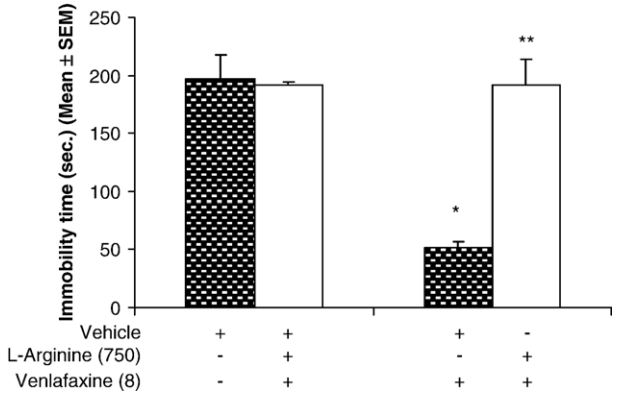

Fig. 2. Effect of venlafaxine (8 mg/kg, i.p.) and its modification by L-arginine (750 mg/kg, i.p.) on the mean immobility period in mouse FST. L-arginine was administered 30 min before the treatment with venlafaxine and after further 30 min, animals were challenged with FST. The values are expressed as mean± S.E.M. (n=6–8). Data was analysed by Two-Way Analysis of Variance (ANOVA) followed by Dunnett's test. *P<0.001 compared with the vehicle-treated control; **P<0.001 compared with the same group pretreated with vehicle.