European Journal of Pharmacology 589 (2008) 163–172

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

On the mechanism of antidepressant-like action of berberine chloride

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ARTICLE INFO

Article history:
Received 4 February 2008
Received in revised form 25 April 2008
Accepted 19 May 2008
Available online 3 June 2008

Keywords:
Berberine chloride
Antidepressant
Nitric oxide
Sigma receptors
Monoamines

ABSTRACT

Berberine, an alkaloid isolated from *Berberis aristata* Linn. has been used in the Indian system of medicines as a stomachic, bitter tonic, antiamoebic and also in the treatment of oriental sores. Evidences have demonstrated that berberine possesses central nervous system activities, particularly the ability to inhibit monoamine oxidase-A, an enzyme involved in the degradation of norepinephrine and serotonin (5-HT). With this background, the present study was carried out to elucidate the antidepressant-like effect of berberine chloride in different behavioural paradigms of despair. Berberine (5, 10, 20 mg/kg, i.p.) inhibited the immobility period in mice in both forced swim and tail-suspension test, however, the effect was not dose-dependent. Berberine (5 and 10 mg/kg, i.p.) also reversed the reserpine-induced behavioral despair. Berberine (5 mg/kg, i.p.) enhanced the anti-immobility effect of subeffective doses of various typical but not atypical antidepressant drugs in forced swim test. Berberine (5 mg/kg, i.p.) following its acute administration in mice resulted in increased levels of norepinephrine (31%), serotonin (47%) and dopamine (31%) in the whole brain. Chronic administration of berberine (5 mg/kg, i.p.) for 15 days significantly increased the levels of norepinephrine (29%), serotonin (19%) as well as dopamine (52%) but at higher dose (10 mg/kg, i.p.), there was no change in the norepinephrine (12%) levels but a significant increase in the serotonin (53%) and dopamine (31%) levels was found. The antidepressant-like effect of berberine (5 mg/kg, i.p.) in forced swim test was prevented by pretreatment with L-arginine (750 mg/kg, i.p.) or sildenafil (5 mg/kg, i.p.). On the contrary, pretreatment of mice with 7-nitroindazole (7-NI) (25 mg/kg, i.p.) or methylene blue (10 mg/kg, i.p.) potentiated the effect of berberine (2 mg/kg, i.p.) in the forced swim test. Pretreatment of mice with (+)-pentazocine (2.5 mg/kg, i.p.), a high-affinity sigma1 receptor agonist, produced synergism with subeffective dose of berberine (2 mg/kg, i.p.). Pretreatment with various sigma receptor antagonists *viz.* progesterone (10 mg/kg, s.c.), rimcazole (5 mg/kg, i.p.) and *N*-[2-(3,4-dichlorophenyl)ethyl]-*N*-methyl-2-(dimethylamino) ethylamine (BD1047; 1 mg/kg, i.p.) reversed the anti-immobility effects of berberine (5 mg/kg, i.p.). Berberine at lower dose did not affect the locomotor activity and barbiturate-induced sleep time. It produced mild hypothermic action in rats and displayed analgesic effect in mice. Taken together, theses findings demonstrate that berberine exerted antidepressant-like effect in various behavioural paradigms of despair possibly by modulating brain biogenic amines (norepinephrine, serotonin and dopamine). Further, nitric oxide pathway and/or sigma receptors are involved in mediating its antidepressant-like activity in mouse forced swim test.

1. Introduction

Berberine is a plant alkaloid with a long history of medicinal use in both Ayurvedic and Chinese medicine. The various plant sources of berberine include *Hydrastis canadensis* (goldenseal), *Coptis chinensis* (Coptis or goldenthread), *Berberis aquifolium* (Oregon grape), *Berberis vulgaris* (barberry), and *Berberis aristata* (tree turmeric). It has been reported to possess multiple pharmacological effects (Zeng et al., 2003; Birdsall and Kelly, 1997; Kulkarni et al., 1972; Huang et al., 1991) and hold promising properties as a drug for diabetes (Lee et al., 2006), hyperlipemia (Chang and But, 1986), coronary artery disease (Kong et al., 2004), hypertension (Kong et al., 2004), ischemic stroke (Birdsall and Kelly, 1997), neurodegenerative and neuropsychiatric disorders (Zhu and Qian, 2006; Yoo et al., 2006; Peng et al., 2004). Berberine is reported to have inhibitory action on monoamine oxidase enzyme particularly monoamine oxidase-A isoform with an IC50 values of 126 μM (Kong et al., 2001). It is well documented that monoamine oxidase inhibitors increase the concentrations of norepinephrine, serotonin (5-HT) and dopamine in the brain and have antidepressant effects (Bryant and Brown, 1986; Kanazawa, 1994). Recently, in one of the studies, berberine is shown to have antidepressant-like effect in forced swim and tail-suspension test (Peng et al., 2007). However, the

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doi:10.1016/j.ejphar.2008.05.043

mechanism of its antidepressant action is not fully clear which encouraged us to investigate the effects of berberine in various animal models of depression.

Besides biogenic amine (noradrenergic and serotonergic) theory of mental depression, multiple neurotransmitter and receptor systems are involved in mediating the effect of various antidepressants. Recently, it has been shown that L-arginine-nitric oxide-cyclic guanosine monophosphate 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, synthesized from L-arginine by nitric oxide synthase, and has been implicated in neurotransmission, synaptic plasticity, learning, perception of pain, aggression and depression (Esplugues, 2002). Recent evidences have shown that the reduction of nitric oxide levels within the hippocampus can induce antidepressant-like effects, thus implicating endogenous hippocampal nitric oxide in the neurobiology of stress and depression (Joca and Guimaraes, 2006). Nitric oxide is also known to modulate the levels of cyclic guanosine monophosphate which in turn known to produce depression like state in animals (Kaster et al., 2005). The antidepressant drugs showed enhanced efficacy when the nitric oxide synthase enzyme was inhibited (Harkin et al., 2004). Inhibitors of nitric oxide synthase and methylene blue increased extracellular levels of serotonin and dopamine in the rat ventral hippocampus and L-arginine had opposite effect, thus demonstrating the role of endogenous nitric oxide in the regulation of serotonin and dopamine levels in the hippocampus (Wegener et al., 2003). Recently, we have shown the involvement of L-arginine–nitric oxide–cyclic guanosine monophosphate pathway in the antidepressant activity of bupropion, a dopamine reuptake inhibitor (Dhir and Kulkarni, 2007a).

Sigma receptors have recently been the targets for the development of psychotropic drugs used in schizophrenia, depression and Alzheimer's (Ishihara and Sasa, 2002). Out of the established two isoforms of sigma receptors (subtypes sigma1 and sigma2), sigma1 receptors are particularly involved in learning and memory, response to stress and depression, psychostimulant-induced sensitization, vulnerability to addiction and pain perception (Hashimoto and Ishiwata, 2006). Sigma1 receptor agonists when administered to mice or rats modulated the release of serotonin and dopamine in the brain (Bermack and Debonnel, 2005; Kobayashi et al., 1997). Further, it was suggested that stimulation of sigma1 receptors alleviated behavioral despair in both forced swim (Skuza and Rogoz, 2006) and tail-suspension test (Ukai et al., 1998). Sigma receptors may play, in some way, a role in the antidepressant actions of selective serotonin reuptake inhibitors such as fluoxetine, fluvoxamine or escitalopram (Narita et al., 1996). One of the recent studies from our laboratory has indicated the involvement of sigma1 receptors in modulating the antidepressant-like effect of neurosteroids in mouse forced swim (Reddy et al., 1998) or tail-suspension test (Dhir and Kulkarni, 2008) and more recently we have demonstrated the involvement of sigma1 receptors in the antidepressant action of venlafaxine (dual reuptake inhibitors of serotonin and norepinephrine) (Dhir and Kulkarni, 2007b). As sigma1 receptors are known to affect the release of catecholamines, affect the outcome of antidepressants, it was hypothetized that sigma receptor modulation may be involved in the antidepressant-like action of berberine.

With this background, the present study was undertaken to further establish the antidepressant-like action of berberine in various behavioral paradigms of despair and also to study its interaction with different doses of fluoxetine or tranylcypromine. Other behavioral effects of the drug such as its effect on locomotion, sleep time, pain perception and alteration in body temperature were also noted. The study was further extended to observe the chronic (for 15 days) effect of berberine chloride in mouse forced swim test and the associated neurochemical alterations. The possible involvement of central sigma1 receptors in the antidepressant-like action of berberine was also investigated in mouse forced swim test. This was examined by interactive studies using (+)-pentazocine (a sigma1 receptor agonist), progesterone (a sigma1 receptor antagonist neurosteroid), rimcazole (another sigma1 receptor antagonist) or *N*-[2-(3,4-dichlorophenyl)ethyl]-*N*-methyl-2-(dimethylamino) ethylamine (BD1047; a novel sigma1 receptor antagonist), respectively.

2. Materials and methods

2.1. Animals

Male albino mice (Laca strain) weighing between 22 and 30 g bred in Central Animal House (CAH) facility of the Panjab University, Chandigarh were used. For measurement of rectal temperature, male albino Wistar rats weighing between 150 and 200 g were used. The animals were housed under a 12-h/12-h light and dark cycle (lights on at 0700 h), with standard laboratory conditions 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 Guidelines (INSA) for the use and care of experimental animals.

2.2. Experimental protocol

2.2.1. Forced swim test

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; Kulkarni and Dhir, 2007; Dhir and Kulkarni, 2007a,b). 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 an 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 (Porsolt et al., 1978). The total immobility period for a period of 6 min was recorded with the help of stop-watch (Kulkarni and Mehta, 1985).

2.2.2. Tail-suspension test

The test procedure was carried out according to the previously standardized and validated animal model in our laboratory (Kulkarni and Dhir, 2007; Dhir and Kulkarni, 2008). In brief, mice were individually suspended on the edge of a lever above (58 cm) the table top by using adhesive tape placed approximately 1 cm from the tip of the tail. The other side of the lever had a writing pen to record the activity on the moving drum. The duration of immobility period was recorded on a moving drum (speed previously standardized) rotating at a speed of 15 cm/min (Kulkarni and Dhir, 2007; Dhir and Kulkarni, 2008). A mouse was considered immobile when it hangs passively and completely motionless. The total immobility was measured for a period of six minutes (Steru et al., 1985).

2.2.3. Reserpine-induced behavioral despair in mice

The procedure was conducted according to a previously validated method in our laboratory (Shaji and Kulkarni, 1988; Dhir and Kulkarni, 2007a). In brief, reserpine (2 mg/kg, i.p.) was administered 4 h before challenging the animal to forced swimming as discussed above (Shaji and Kulkarni, 1988; Dhir and Kulkarni, 2007a). Berberine was administered in different doses in reserpinised mice 60 min before recording the immobility period. The immobility period was recorded for total of six minutes.

2.2.4. Body temperature measurement in rats

Variation in rectal temperature was recorded using a telethermometer (Yellow Springs Instrument Co., Inc. USA) by inserting the thermister probe to a depth of 2.5 cm into the rectum of rat. The rectal