

Protective Effect of BR-16A, a Polyherbal Preparation against Social Isolation Stress: Possible GABAergic Mechanism

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The antistress effects of BR-16A, a polyherbal preparation and its interaction with GABAergic modulators against social isolation-induced stress were investigated in the present study. Isolation stress was induced by keeping the mice (Laca strain) individually in each cage for 3 weeks and various drug treatments were given for a period of 5 days before the start of the experiments. The various behavioural parameters examined included pentobarbitone-induced sleep (sleep latency and duration), analgesia (tail-flick test) and locomotor activity, respectively. BR-16A (100 mg/kg and 200 mg/kg) treatment for 5 days significantly reversed the social isolation stress-induced prolongation of onset and decrease in pentobarbitone-induced sleep, increased total motor activity and stress-induced antinociception. When diazepam (0.5 mg/kg), a benzodiazepine agonist, was co-administered with BR-16A (100 mg/kg), it significantly potentiated the reversal of pentobarbitone-induced shortening of sleep time effects; increased locomotor activity and stress induced antinociceptive effects. However, the sleep latency was not decreased significantly. Further, flumazenil (2 mg/kg), a benzodiazepine receptor antagonist and FG 7142 (10 mg/kg), an inverse agonist, when co-administered with BR-16A (100 mg/kg), showed no significant reversal on pentobarbitone-induced hypnosis, locomotor activity and social isolation-induced antinociception compared with their effects *per se*. The present study demonstrated the antistress effects of BR-16A preparation against social isolation-induced stress. The present study also suggests that the GABAergic system may be involved in its antistress effect. Copyright © 2006 John Wiley & Sons, Ltd.

Keywords: stress; social-isolation stress; GABA; BR-16A; sleep; diazepam.

INTRODUCTION

Loneliness or lack of companionship may cause a psychological experience of social isolation in human beings (Steptoe *et al.*, 2004). Constant or continuous isolation (stress) is one of the causes of insomnia and sleep related-disorders including behavioural and endocrine (Esch, 2002; Jones *et al.*, 2001; Imaki *et al.*, 1986). In animals also physiological stress is known to activate the adenohipophyseal axis (HPA) and to alter behavioural responses and drug effects. Reports are available that clearly indicate that stress modulated the arousal level in animals (Ojima *et al.*, 1997; Matsumoto *et al.*, 1997). GABAergic neurotransmission is thought to play an important role in stress and stress-related disorders (Dong *et al.*, 1999a; Dong *et al.*, 1999b; Saito *et al.*, 2002; Martijena *et al.*, 2002). GABA_A receptor modulators are widely used as antistress drugs (Ojima *et al.*, 1997; Dong *et al.*, 1999b).

BR-16A is a polyherbal psychotropic preparation containing the following active ingredients (mg): *Bacopa monnieri* 216, *Centella asiatica* 70, *Withania somnifera* 52, *Evolvulus alsinoides* 52, *Nardostachys jatamansi* 52, *Valeriana wallichii* 50, *Embelia ribes* 50, *Prunus amygdalus* 50, *Acorus calamus* 42, *Tinospora cordifolia* 36, *Terminalia chebula* 36, *Emblica officinalis* 36,

Oroxylum indicum 32, *Celastrus paniculatus* 32, *Bacopa monnieri* 80, *Mucuna pruriens* 18, *Elettaria cardamomum* 18, *Terminalia arjuna* 18, *Foeniculum vulgare* 18, *Ipomoea digitata* 18, *Zingiber officinale* 14, *Terminalia belerica* 14, *Myristica fragrans* 14, *Syzygium aromaticum* 10, *Mukta pishti* (pearl powder) 3. These constituents are well known to be used in the ancient system of Ayurvedic medicine for the management of nervous disorders (Chauhan *et al.*, 1993; Verma and Kulkarni, 1991). It is a safe preparation and the LD₅₀ has been reported to be 2400 mg/kg in mice by the oral route of administration (personal communication). The clinical trials with this preparation have shown it to be effective in cases of mental retardation, cerebral deficit and behavioural disturbances in mentally retarded children (Dave *et al.*, 1993).

The objective of the present work was to evaluate the effectiveness of BR-16A preparation in social isolation-induced stress in mice and its interaction with GABA_A/BZ receptor modulators to understand the possible mechanism of action.

MATERIAL AND METHODS

Laca mice (20–25 g) of either sex bred in the Central Animal House facility of Panjab University were used. Animals of either sex (male and female) were used to avoid any gender bias on the effect of the preparation. Moreover, BR-16A is meant to be used both in male

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and female individuals. The animals were housed under a standard light/dark cycle with food and water provided *ad libitum*. Animals were acclimatized to laboratory conditions before test. Each animal was used once in the experiment. The experiments were performed between 09.00 and 17.00 h. The experimental protocols were approved by the Institutional Animal Ethics Committee and were conducted according to the Indian National Science Academy Guidelines for the use and care of experimental animals.

Social isolation stress. Animals were housed individually (socially isolated) for 3 weeks and drug treatment was given for a period of 5 days before the start of the experiment. Animals were acclimatized to laboratory conditions before test. The animals were divided into different groups consisting of five animals.

Pentobarbitone-induced sleep. Pentobarbitone-induced hypnosis is one of the animal models used to test the sleep promoting or sedative properties of a drug. Two parameters were used to assess the sleep promoting effect, i.e. sleep latency (onset of sleep) and total sleep time (duration of sleep).

Locomotor activity. The total locomotor activity was assessed prior to subjecting the animals to immobilization stress or the effect of various treatments alone or in combination. The locomotor activity, ambulatory and total activity, was measured using an actophotometer and the score was expressed in terms of total counts/5 min per animal.

Antinociceptive effect. The nociceptive threshold was determined following the tail-flick method as described earlier (Kulkarni, 1980). Baseline latencies to tail-flick withdrawal from the radiant heat source (3–5 s) were established. A cut-off time of 10 s was observed to prevent any injury to the tail.

Drugs, dose and treatment. BR-16A (100, 200 mg/kg, p.o.), diazepam (0.5 mg/kg, i.p.), flumazenil (2 mg/kg, i.p.) and pentobarbitone sodium (45 mg/kg), and FG 7142 (10 mg/kg i.p.) were used. Flumazenil was dissolved with a few drops of DMSO and then was made up to volume with distilled water. FG 7142 was suspended in 0.5% carboxy methylcellulose. Drug treatment was given for a period of 5 days to animals subjected to isolation stress.

Statistical analysis. The results were expressed as mean \pm SEM and analysed by using one-way analysis of variance (ANOVA) followed by Dunnett's test. In all the tests, the criterion for statistical significance was $p < 0.05$.

RESULTS AND DISCUSSION

Effects of BR-16A preparation and its modification by GABA_A-ergic agents on pentobarbitone (PB)-induced sleep time in social isolation-induced stress in mice

As shown in Fig. 1, the prolongation of sleep latency and shortening of duration of sleep due to PB occurred

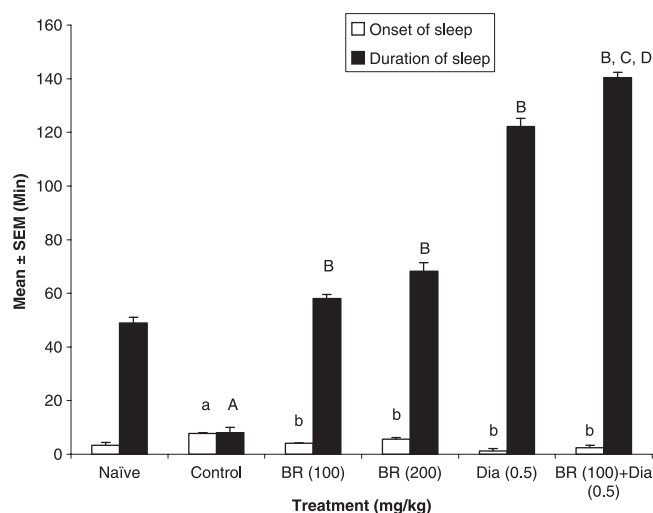


Figure 1. Effect of BR-16A preparation and its modification by diazepam (Dia) on pentobarbitone (PB)-induced sleep (onset of action and duration of action) time in social isolation stress in mice. Values are expressed as mean \pm SEM. ^{a, A} $p < 0.05$ compared with naïve mice, ^{b, B} $p < 0.05$ compared with control (isolation stress), ^c $p < 0.05$ compared with Dia (5), ^D $p < 0.05$ compared with BR-16A (100) (ANOVA followed by Dunnett's test).

in socially isolated mice compared with naïve mice (group housed mice). BR-16A (100 mg/kg and 200 mg/kg) treatment for 5 days caused a significant ($p < 0.05$) reversal of the social isolation stress-induced prolongation of onset and decrease in PB-induced sleep. When diazepam (0.5 mg/kg) was co-administered with BR-16A (100 mg/kg), a significant potentiation of reversal of PB-induced shortening of sleep time was observed. However, the sleep latency was not decreased significantly (Fig. 1). Flumazenil, a benzodiazepine receptor antagonist (2 mg/kg), when co-administered with BR-16A (100 mg/kg), did not reverse the PB-induced shortening of sleep latency or the prolongation of sleep duration. Similarly, with FG 7142, an inverse agonist when co-administered with BR-16A (100 mg/kg), no significant reversal of PB effects were observed compared with the effect *per se* in social isolation-induced stress (Fig. 2). However, the effects were significant when compared with the control (non-stressed) animals.

Effect of BR-16A preparation and its modification by GABA_A-ergic agents on locomotor activity in social isolation-induced stress in mice

As shown in Fig. 3, a significant increase in total motor activity (ambulation and rearing) was observed in socially isolated mice compared with naïve mice (group housed mice). BR-16A (100, 200 mg/kg) treatment for 5 days caused a significant reversal of the increase in total motor activity. The effects were significant compared with the control ($p < 0.05$). When diazepam (0.5 mg/kg) was co-administered with BR-16A (100 mg/kg), a further reversal of increase in locomotor activity was observed. As shown in Fig. 3, flumazenil (2 mg/kg) and FG 7142, treatments failed to produce any significant effect on the BR-16A (100 mg/kg)-induced locomotor activities.

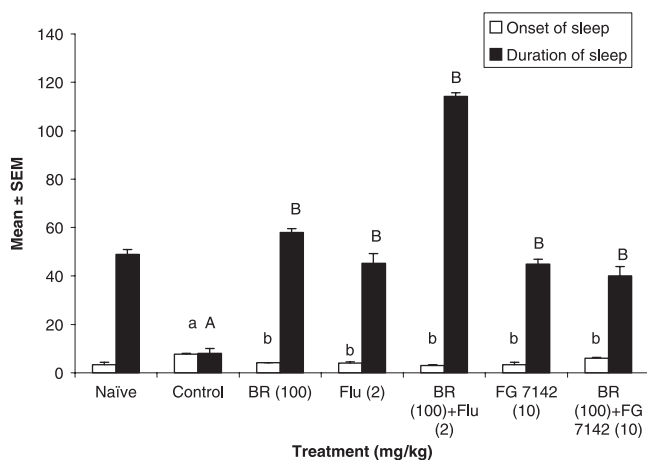


Figure 2. Effect of BR-16A preparation on the modulation of pentobarbitone-induced sleep (onset of action and duration of sleep) by flumazenil (Flu) and FG 7142 in social isolation stress in mice. Values are expressed as mean \pm SEM. ^a $p < 0.05$ compared with naïve mice, ^b $p < 0.05$ compared with control (isolation stress) (ANOVA followed by Dunnett's test).

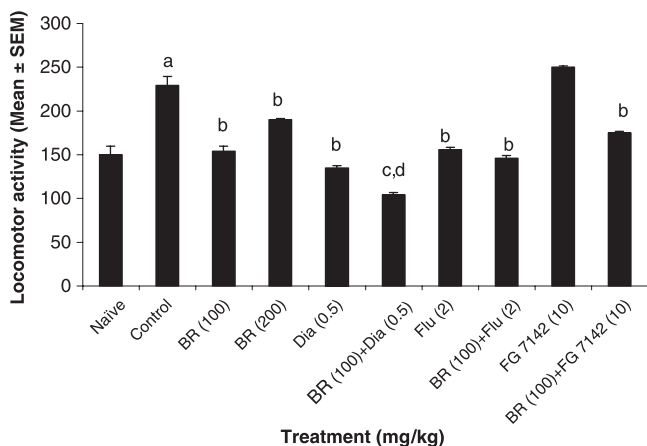


Figure 3. Effect of BR-16A preparation and its modification by diazepam (Dia), flumazenil (Flu) and FG 7142 on locomotor activity in social isolation stress in mice. Values are expressed as mean \pm SEM. ^a $p < 0.05$ compared with naïve mice, ^b $p < 0.05$ compared with control (isolation stress), ^c $p < 0.05$ compared with Dia (0.5), ^d $p < 0.05$ compared with BR-16A (100) (ANOVA followed by Dunnett's test).

Effect of BR-16A preparation and its modification by GABA_A-ergic agents on analgesic activity in social isolation-stress in mice

As shown in Fig. 4, a significant increase in the threshold to pain (antinociceptive effect) was observed in socially isolated mice compared with naïve mice (group housed mice). BR-16A (100, 200 mg/kg) treatment significantly reversed the social isolation stress-induced percentage increase of analgesia compared with the control (stressed mice) ($p < 0.05$). The effect was dose dependent. When diazepam (0.5 mg/kg) was co-administered with BR-16A (100 mg/kg), a further reversal of antinociceptive effects was noticed that was statistically significant compared with the effect *per se* (Fig. 4). Flumazenil (2 mg/kg) and FG 7142 treatments did not produce any significant effect on the analgesic activity of the BR-16A preparations (5 mg/kg and 100 mg/kg)

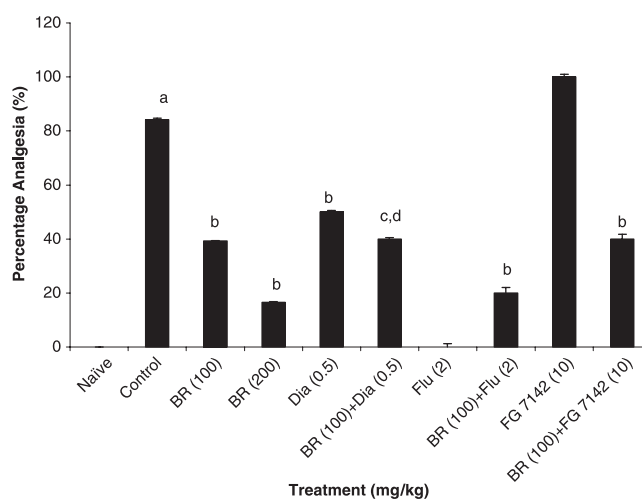


Figure 4. Effect of BR-16A preparation and its modification by diazepam (Dia), flumazenil (Flu) and FG 7142 on analgesic activity in social isolation stress in mice. Values are expressed as mean \pm SEM. ^a $p < 0.05$ compared with naïve mice, ^b $p < 0.05$ compared with control (isolation stress), ^c $p < 0.05$ compared with Dia (0.5), ^d $p < 0.05$ compared with BR-16A (100) (ANOVA followed by Dunnett's test).

compared with their effect *per se*. However, their individual effect was significant compared with the control.

In the present study, the antistress effects of BR-16A preparation on behaviour, and its interaction with GABA_Aergic modulators were investigated. GABA_Aergic neurotransmission is thought to play an important role in the modulation of the central response to stress and is presumed to have a major role in the mediation of emotional behaviour. It has been reported that endogenous substances with an inverse BZD receptor agonist-like property are involved in the hypnotic activity of pentobarbital following social isolation stress (Ojima *et al.*, 1997). Recent evidence demonstrates a link between GABA_Aergic synaptic activity and CRF neurons in the hypothalamic paraventricular nucleus (Meister *et al.*, 1988), and GABA reportedly inhibits the stimulated release of CRF from the hypothalamic tissues *in vitro* (Calogero *et al.*, 1988). In addition, CRF-induced anxiogenic behavioural effects and decrease in PB sleep in rodents have been shown to be attenuated by the steroidal GABA_A agonist alphaxalone (Britton *et al.*, 1992). The above interaction indicates a positive interaction between the GABA_A and CRF system. The present results demonstrated that BR-16A suppressed the increase in the arousal level of mice subjected to social isolation stress. BR-16A preparation significantly decreased the sleep latency and sleep duration due to PB, increased motor activity, and antinociceptive effect in social isolation-stressed mice. Diazepam, a positive modulator of the GABA_A receptor system, potentiated the effects of the BR-16A (100 mg/kg) preparation. This suggests that the GABA_Aergic receptor system is involved in the behavioural changes induced by social isolation stress. This contention is supported by the observations (Dong *et al.*, 1999b; Martijena *et al.*, 2002) that a reduced GABAergic inhibitory or down-regulation of GABA_A receptor control could be implicated in the emotional sequelae generated by an uncontrollable stressor and that the suppression of this reduction seems to be associated with the occurrence

of behavioural disorders. These observations suggest that the BR-16A brings about its behavioural effects through the GABA_Aergic system and when co-administered with GABA_Aergic agents, its effects are potentiated.

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