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A novel 5-HT_{2A} receptor antagonist exhibits antidepressant-like effects in a battery of rodent behavioural assays: Approaching early-onset antidepressants

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ABSTRACT

Collective evidence suggests that inhibition of neuronal 5-hydroxytryptamine type 2A (5-HT_{2A}) receptors contributes to the assuagement of depression-like behaviour in rodents. The present study evaluated the antidepressant-like effect of the 5-((4-benzo [α] isothiazol-3-yl) piperazin-1-yl) methyl)-6-chloroindolin-2-one (BIP-1), a compound having affinity to 5-HT_{2A} receptors, using a rodent behavioural test battery. Acute BIP-1 (0.25–4 mg/kg) pretreatment reduced the quipazine-induced head twitches in mice and produced antidepressant-like effects in mouse forced swim and tail suspension tests. BIP-1 reversed the depressogenic-like effects of meta-chlorophenyl piperazine and augmented the antidepressant-like effects of amitryptiline and harmane. Chronic (14 days) treatment with BIP-1 (1 and 2 mg/kg) or amitriptyline (10 mg/kg) alleviated the behavioural anomalies of olfactory bulbectomised rats in modified open field exploration, social interaction, hyperemotionality and sucrose preference paradigms. When BIP-1 treatment was combined with amitryptyline, a short duration regimen (7 days) was sufficient to reverse the bulbectomy induced anomalies. This investigation revealed that 5-HT_{2A} receptor antagonism is the principal mechanism behind the antidepressant-like effects of BIP-1. Finally, we propound the combination of 5-HT_{2A} receptor antagonists and tricyclic antidepressants as a likely strategy to achieve an early-onset of antidepressant action.

1. Introduction

The identification of neuronal metabotropic 5-hydroxytryptamine type 2A (5-HT_{2A}) receptors in the putative correlates of depression, namely, hippocampus, amygdala, prelimbic prefrontal cortex, striatum and olfactory structures (Xu and Pandey, 2000; Miner et al., 2003; McDonald and Mascagni, 2007) has strengthened the role of these receptors in rodent depression-like behaviour and in human depression. Supersensitivity of 5-HT_{2A} receptors (present in both platelets and brain tissue) (Van Oekelen et al., 2003) and an increased number of 5-HT_{2A} receptor binding sites in the brain have been observed in depressed patients (Yates et al., 1990; Shelton et al., 2009) and suicide victims (Stanley and Mann, 1983; Mann et al, 1986; Arora and Meltzer, 1989; Rosel et al., 2004). The paradoxical, antagonist-induced desensitisation and downregulation of 5-HT_{2A} receptors (Van Oekelen et al., 2001; Gray and Roth 2001; Hanley and Hensler, 2002) and the regulatory effects of this receptor on brain derived neurotropic factor pathway (Vaidya et al., 1997; Vaidya et al., 1999; Rios et al., 2006) are known to have interesting implications in the etiology of depression.

A multitude of neuropsychopharmacological investigations have associated 5-HT_{2A} receptor modulation and depression-like behaviour which are mentioned below. In general, antidepressant treatment downregulates 5-HT_{2A} receptors (Peroutka and Snyder, 1980; Blackshear and Sanders-Bush, 1982; Goodwin et al., 1984; Cross and Horton, 1988; Yamauchi et al., 2006) and it has been speculated that selective downregulation of 5-HT_{2A} receptors by itself produces antidepressant-like effects (Sibille et al., 1997; Celada et al., 2004). In addition, rodent assays (behavioural/electrophysiological) have demonstrated antidepressant-like effects of high affinity 5-HT_{2A} receptor antagonists namely LY367265 (Pullar et al., 2000), M100907 (Marek et al., 2001; Marek et al., 2005), EMD-281014 (Patel et al., 2004) and Wf-516 (El Mansari and Blier, 2008). 5-HT_{2A} receptor antagonism is the salient mechanism behind the clinical efficacy of serotonin antagonist and reuptake inhibitiors (SARI), trazodone and nefazodone (Taylor et al., 1995; Macs et al., 1996; Smeraldi et al., 1997; Davis et al., 1997; Ulug et al., 2001; Dursun et al., 2002; Gelenberg et al., 2003) and the noradrenergic and specific serotonergic antidepressant, mirtazapine (Fawcett and Barkin, 1998; Anttila and Leinonen, 2001). Recent reports indicate a comparable efficacy of the aforementioned drugs to that of selective serotonin

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reuptake inhibitors (SSRIs) (Papakostas and Fava, 2007; Papakostas et al., 2008). Futhermore, 5-HT_{2A} receptor antgonists augment the SSRI induced cortical dopamine and norepinephrine release (Szabo and Blier, 2002; Huang et al., 2006) which suggests the use of this combination as one of the approaches to manage treatment-resistant depression (Macs et al., 1996; Ostroff and Nelson, 1999; Shelton et al., 2001; Marek et al., 2005).

A novel series of piprazinylphenanthridine derivatives with high affinity to 5-HT_{2A} and D_2 receptors, was originally designed and synthesised as potential atypical antipsychotics (Howard et al., 1996). The 6-chloro derivative, 5-((4-benzo [α] isothiazol-3-yl) piperazin-1-yl)methyl)-6-chloroindolin-2-one (BIP-1) was found to possess a significant K_i in 5-HT_{2A} receptor binding assay and an optimum Log *P* value. For the scheme of synthesis and details of receptor binding assay see supplementary material. Interestingly, a pilot study (Table 1) indicated that BIP-1 possessed significant antidepressant-like effects in mouse forced swim test (FST) compared to other compounds of the series. This finding inspired us to further examine the antidepressant-like effects of BIP-1, utilising a battery of behavioural assays (Pandey et al., 2008; Rajkumar et al., 2009). The test battery comprised of predictive assays namely FST (Porsolt et al. 1977; Bourin et al. 2002) and tail suspension test (TST, Steru et al. 1985) in mice and reversal of olfactory bulbectomy (OBX) induced behavioural anomalies in rats (Cairncross et al., 1979; Song and Leonard 2005). Effect of BIP-1 pretreatment on the quipazine (selective 5-HT_{2A} agonist) induced head twitches in mice and interaction studies (in mice FST) with mCPP (5-HT_{2A} receptor agonist), 8-OH-DPAT (5-HT_{1A} receptor agonist), harmane (MAO inhibitor) and amitriptyline (trycyclic antidepressant) were carried out to elucidate the probable mechanism underlying the antidepressant-like effects. Besides, the effects of a shorter duration (7 days) of BIP-1 treatment on the behaviour of bulbectomised rats were evaluated to detect an early-onset of antidepressant-like effects.

2. Materials and methods

2.1. Animals

Male Albino mice (25–35 g) and male Wistar rats (230–280 g) were obtained from Hissar Agricultural University, Hissar, Haryana, India. Animal experimentation was conducted in adherence to the Institutional Animal Ethics Committee of Birla Institute of Technology & Science, Pilani, India (Protocol No. IAEC/RES/4/1, dated 22.09.04 and IAEC/RES/7/1, dated 24.04.06). The animals were housed in laboratory cages and maintained under standard light (lights on from 7:00 A.M. to 7.00 P.M.), temperature (23 ± 2°C), and humidity (50–60%) conditions in the housing unit for at least 1 week before the commencement of experiments. Each treatment group consisted of 6–8 randomly chosen animals. The animals were given free access to food (standard pellet feed) and filtered water. In order to prevent habituation effects the animals were used only once for each experiment.

Table 1
Effects of different compounds of the BIP series and AMI on the duration of immobility and swimming behaviour of mice in forced swim test.

Treatment	Dose	Duration of immobility (s)	Number of quadrants crossed
Vehicle	10 ml/kg	170.00 ± 5.31	17 ± 1.48
BIP-1 (6-chloro derivative)	1 mg/kg	88.07 ± 6.19*	41.44 ± 2.61*
BIP-2 (3-methyl derivative)	1 mg/kg	150.63 ± 6.44	24.63 ± 2.10
BIP-3 (1- ethyl derivative)	1 mg/kg	135.50 ± 7.56*	22.25 ± 2.30
BIP-4 (unsubstituted)	1 mg/kg	148.63 ± 5.24	19.88 ± 1.69
BIP-5 (1 methyl derivative)	1 mg/kg	133.75 ± 8.46*	23.25 ± 2.06
AMI	10 mg/kg	118.24 ± 7.18*	34.38 ± 2.81*

Values are mean ± S.E.M. $n = 8$ per group. *$P < 0.05$ compared with vehicle treatment (ANOVA followed by Dunnett's T3 test). The shaded area indicates the compound that was selected for further investigation.

2.2. Drugs

BIP-1 was synthesised by the Chemistry Group, Birla Institute of Technology & Science. Fluoxetine (FLX) hydrochloride and paroxetine (PAR) hydrochloride hemihydrate were obtained as gift samples from IPCA labs, Mumbai, India. Amitriptyline (AMI) hydrochloride and venlafaxine (VLF) hydrochloride were gifts from Ranbaxy Research Laboratories, Grugaon, India. Ketanserin (KET) tartrate and quipazine were procured from Sigma Aldrich Chemicals Private Limited, New Delhi. 8-Hydroxy dipropylaminotetralin (8-OH-DPAT) and harmane (HAR) hydrochloride were obtained from Tocris Chemicals, UK. Meta-chlorophenyl piperazine (mCPP) was obtained from Lancaster Chemicals, USA. Ketamine and xylazine were obtained from Neon Laboratories Ltd. and Indian Immunologicals, India, respectively. All other chemicals used in the study were of analytical grade. BIP-1 was solubilised in 10% polyethylene glycol (PEG) and all other drugs were dissolved in sterile distilled water. The drugs were freshly prepared and the unused portions of drug solutions were discarded after 24 h.

2.3. Treatment schedule

In the acute dose–response study, the animals received a single intraperitoneal (i.p.) injection of BIP-1 (0.25, 0.5, 1, 2 or 4 mg/kg) and 30 min after the dose administration they were subjected to FST or TST. Based on the results from the acute study, one dose level of BIP-1 was selected for interaction studies (in FST) with conventional antidepressants/ligands. In interaction studies, BIP-1 and interacting agents were administered (i.p.) 45 and 30 min respectively, before behavioural testing (specified otherwise) as per the previously reported protocols (Redrobe and Bourin 1997; Bourin et al., 2002; Rajkumar et al., 2009).

The OBX/sham-operated rats, received i.p. injections of vehicle/BIP-1 (1 and 2 mg/kg)/AMI (10 mg/kg) once a day for either 7 or 14 days. In order to avoid the acute effects of drug treatment on the behaviour, the OBX/sham-operated rats were subjected to the assessments 20 h after the last drug/vehicle administration. The drug administration and behavioural assessments were performed between 10:00 and 15:00 h. The doses of standard antidepressants were selected from the studies conducted earlier in our laboratory (Ramamoorthy et al., 2008; Pandey et al., 2008, Rajkumar et al., 2009). The surgery, rehabilitation, treatment and behavioural assessments in the OBX study were done according to a previously reported schedule (Wang et al., 2007, Rajkumar et al., 2009), with substantial modification (Table 2a and b). The behavioural observations were carried out by trained experimenters, who were blind to the treatment.

2.4. Behavioural assessments in mice

2.4.1. Spontaneous locomotor activity

The spontaneous locomotor activity of mice was assessed using the actophotometer (Boissier and Simon, 1965). Mice were individually placed in the centre of the square arena (30 cm × 30 cm) of the actophotmeter. After an initial familiarisation period (2 min), the digital locomotor scores were recorded for the next 10 min. The arena was cleaned with dilute alcohol and dried between trails.

2.4.2. Quipazine induced head twitches

Mice were treated with test compounds/vehicle (i.p.) and placed in an observation chamber which was identical to the home cage and quipazine (5 mg/kg, i.p.) was administered after 30 min. Thirty minutes after quipazine adminstration, the abrupt lateral movements (which is the head twitch response) were counted for a duration of 15 min (Darmani and Gerdes, 1995).

Table 2
Schedule of treatments and behavioural assessments on OBX and sham-operated rats.

(a) Acute study							
Day 0	0th–1st day	1st–14th day	15th–21st day	22th–25th day			
				Behavioural assessments (drug treatment was continued)			
Surgery	Recovery from surgery (continuous care)	Rehabilitation period. (daily observation and handling)	Drug/vehicle treatment. (once a day i.p. administration for 7 days)	Modified open field exploration followed by drug/vehicle treatment	Social interaction paradigm followed by drug/vehicle treatment	Hyperemotionality test followed by drug/vehicle treatment	Sucrose consumption paradigm
(b) Chronic study							
Day 0	0th–1st day	1st–14th day	15th–28th day	29th–32nd day			
				Behavioural assessments (drug treatment was continued)			
Surgery	Recovery from surgery (continuous care)	Rehabilitation period. (daily observation and handling)	Drug/vehicle treatment. (once a day i.p. administration for 14 days)	Modified open field exploration followed by drug/vehicle treatment	Social interaction paradigm followed by drug/vehicle treatment	Hyperemotionality test followed by drug/vehicle treatment	Sucrose consumption paradigm

2.4.3. Forced swim test

The FST was carried out according to Porsolt et al. (1977). Mice were dropped individually into a glass cylinder (height: 30 cm, diameter: 22.5 cm) filled with water (depth:15 cm). The temprature of water was maintained at 23–25 °C. The duration of immobility was recorded during the last 4 min of a 6 min observation period. A mouse was judged to be immobile when it remained floating in an upright position and exhibited only small movements to keep its head above the water level or made other passive movements. The swimming episodes were recorded as the number of quadrants (demarcated at the base of the cylinder) crossed.

2.4.4. Tail suspension test

Mice were individually suspended by the tail to a horizontal bar (distance from floor: 50 cm) using scotch tape (distance from tip of tail was approximately 1 cm). Typically, mice demonstrated several escape-oriented behaviour interspersed with temporally increasing bouts of immobility (Steru et al. 1985; Rodrigues et al. 2002). The duration of immobility (in seconds) during the 6-min test session was recorded.

2.5. Behavioural assessments in olfactory bulbectomised rats

2.5.1. Surgery

Bilateral OBX was performed according to the previously described procedure (Kelly et al., 1997; Ramamoorthy et al., 2008). Rats were anaesthetised with the cocktail of ketamine and xylazine (75 and 5 mg/kg, i.p. respectively). The head of the rat was fixed in a stereotaxic frame (Inco, India) and the skull was exposed by a midline incision. Burr holes (2 mm in diameter) were drilled 8 mm anterior to bregma and 2 mm on either side of the midline at a point corresponding to the posterior margin of the eye. The olfactory bulbs were removed by suction and the dead space were filled with haemostatic sponge. The scalp was sutured and dabbed with antiseptic solution. Sham surgery was carried out in the same way, including piercing of the dura mater, excepting the removal of olfactory bulbs. To prevent infection, the rats were given Sulprim injection (each ml containing 200 and 40 mg of sulphadiazine and trimethoprim respectively), intramuscularly (0.2 ml/300 g) once a day for 3 days post-surgery. The rats were housed in pairs (one sham-operated and one OBX). Following a rehabilitation period of 14 days the OBX/sham-operated rats were treated with vehicle/test compounds once a day for 7 or 14 days and finally subjected to the behavioural tests explained below.

2.5.2. Modified open field exploration

The apparatus consisted of a circular (diameter: 90 cm) arena with 75-cm high aluminum walls. Faint black lines divided the floor of the arena into 10 cm squares. A light bulb (60 W), positioned 90 cm above the base of the arena, was the only source of illumination in the testing room. Each animal was individually placed in the centre of the open field apparatus and the following parameters were noted for 5 min. (1) Horizontal activity: number of squares crossed, which were counted when the hind limbs of the rat moved to the next square. (2) Vertical activity: number of times the rat stood on its hind limbs to explore the area at a higher plane. (3) Defection: number of faecal pellets left by the animal during the observation period (Ramamoorthy et al., 2008). After each test, the apparatus was sprayed with dilute alcohol and wiped thoroughly to eliminate the residual odour.

2.5.3. Social interaction

The apparatus and testing environment were identical to that of the modified open field test, except for a milder illumination (15 W). On the day of test, rat pairs of the same treatment group (but housed in different cages) were placed far away from each other in the open field arena. Thereafter, the social interaction parameters namely the running (towards each other), crawling (under the other rat), probing, grooming and mounting were recorded for 10 min (File and Hyde, 1978). Crossing which represents passive interaction between the animals was also recorded.

2.5.4. Hyperemotionality

The previously reported procedure (Shibata et al., 1984) was adopted with slight modifications. Hyperemotionality of rats was measured by scoring the responses to the following stimuli namely, (1) bite response: the response to a rod presented 4–5 cm in front of the snout, (2) startle response: the response to a stream of air delivered using 10-ml syringe and directed at the dorsum, (3) struggle response: the response to handling with a gloved hand and (4) fight response: the response to pinching of the tail with blunt forceps. The responses were graded as: 0, no reaction; 1, slight; 2, moderate; 3, marked; or 4, extreme response. For each emotional response, the audible vocalisation was also scored and graded as follows, 0, no vocalisation; 1, occasional vocalisation; or 2, marked vocalisation. The vocal score was added to the corresponding emotional response score. The score for emotional response and audible vocalisation was given within 5 min and rats from different treatment groups were observed on the same day. The results were expressed as the sum of individual scores.

2.5.5. Sucrose preference

Rats had free access to both tap water and sucrose solution (1%) for 5 days from the commencement of drug treatment (15th–19th day, Table 2a and b). The position of the 250-mL bottles containing sucrose solution or tap water was alternated each day, to prevent location preference. On the 25th day (for the 7-day regimen group) and the

32nd (for the 14-day regimen group), the sucrose consumption test was performed (4th day of behavioural test) by presenting both sucrose solution and tap water in the morning (10:00 am). The bottles were weighed after 24 h (the next morning). Sucrose preference was calculated as the percentage of sucrose solution ingested relative to the total amount of liquid consumed (Willner et al., 1987).

2.6. Statistical analyses

The data were expressed as mean ± S.E.M. The results were statistically analysed using SPSS software version 11.0. The single treatment studies were analysed using one-way analysis of variance followed by Dunnett's T3 test, the best suited post hoc test to analyse groups with unequal variances. The results of the interaction studies were analysed using two-way analysis of variance and if a difference was observed, a post hoc Sidak test was performed to compare the effect of pretreatment on the treatment. Sidak test provides relatively tighter bounds in comparison to other post hoc tests.

3. Results

3.1. Pilot study: effect of different compounds of the BIP series on the behaviour of mice in FST

One-way analysis of variance showed a significant difference in the duration of immobility ($F(6, 49) = 16.24$, $P < 0.05$) and swimming behaviour ($F(6, 49) = 11.71$, $P < 0.05$) among the various treatments. BIP-1, BIP-3 and BIP-5 at the dose level of 1 mg/kg and AMI (10 mg/kg) exhibited significant decrease in the duration of immobility compared to vehicle treatment. BIP-1 exhibited a superior antidepressant-like effect when compared to the other compounds of the series (Table 1).

3.2. Effects of BIP-1 treatment on SLA, quipazine induced head twitch response and behaviour of mice in FST and TST

BIP-1 (0.25–2 mg/kg) or AMI (10 mg/kg) treatment had meagre influence on baseline locomotion of mice (Fig. 1). The highest tested dose of BIP-1 (4 mg/kg) significantly ($F(6, 49) = 4.75$, $P < 0.05$) reduced the locomotor scores. Pretreatment with BIP-1 (0.5–4 mg/kg), significantly reduced quipazine induced head twitches ($F(6, 49) = 17.46$, $P < 0.05$) compared to vehicle treatment. KET (4 mg/kg), used as the reference drug, significantly reduced head twitch response (Fig. 2). In mouse FST, AMI (10 mg/kg) and BIP-1 (0.5–4 mg/kg) significantly reduced the duration of immobility ($F(6, 49) = 46.29$, $P < 0.05$) and the aforementioned treatments (except 4 mg/kg) increased the swimming behaviour ($F(6, 49) = 15.57$, $P < 0.05$) compared to vehicle treatment (Fig. 3a and b). A similar effect was observed in the mouse TST (Fig. 3c), wherein BIP-1 (0.5–4 mg/kg) significantly decreased the duration of immobility ($F(6, 49) = 52.16$, $P < 0.05$).

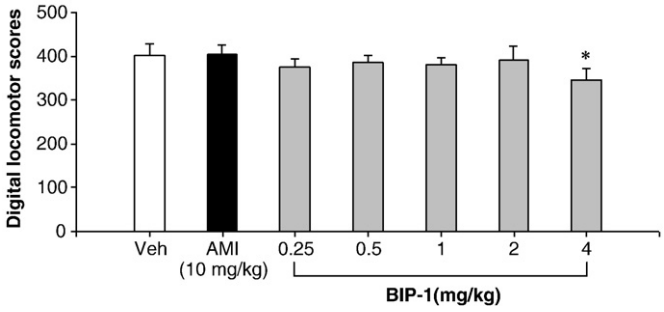

Fig. 1. Effects of AMI and BIP-1 on the spontaneous locomotor activity of mice. The columns represent mean of locomotor scores recorded during a 10 min observation period. Error bars indicate S.E.M. $n = 8$ per group. *$P < 0.05$ compared with vehicle (Veh) treatment (ANOVA followed by Dunnett's T3 test).

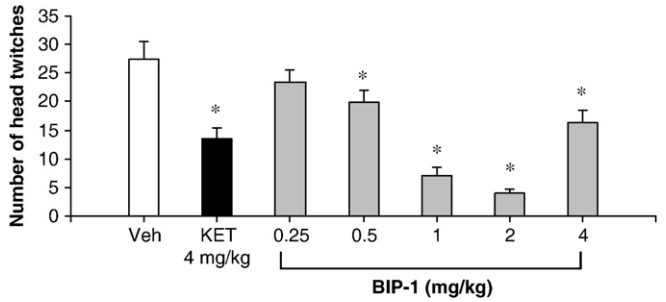

Fig. 2. Effects of KET and BIP-1 on quipazine induced head twitch response in mice. The columns represent mean of number of head twitches recorded during a 15 min observation period. Error bars indicate S.E.M. $n = 8$ per group. *$P < 0.05$ compared with vehicle (Veh) treatment (ANOVA followed by Dunnett's T3 test).

3.3. Effect of BIP-1 on mCPP and 8-OH-DPAT pretreated mice

mCPP (1 mg/kg) treatment significantly increased the duration of immobility ($F(7, 56) = 56.58$, $P < 0.05$) and decreased swimming behaviour ($F(7, 56) = 20.66$, $P < 0.05$) compared to vehicle treatment. On comparing the effects of BIP-1 treatment with mCPP + BIP-1 combined

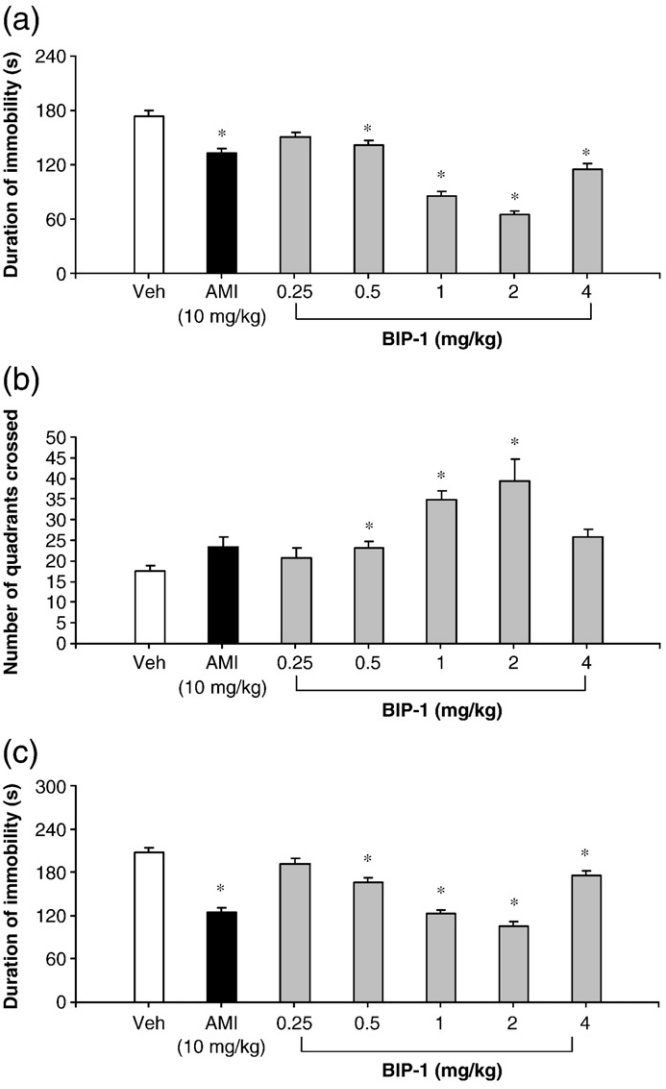

Fig. 3. Effects of AMI and BIP-1 on (a) duration of immobility and (b) swimming behaviour in mice FST and (c) duration of immobility in mice TST. Columns represent mean of values. Error bars indicate S.E.M. $n = 8$ per dose. *$P < 0.05$ compared with vehicle (Veh) treatment (ANOVA followed by Dunnett's T3 test).

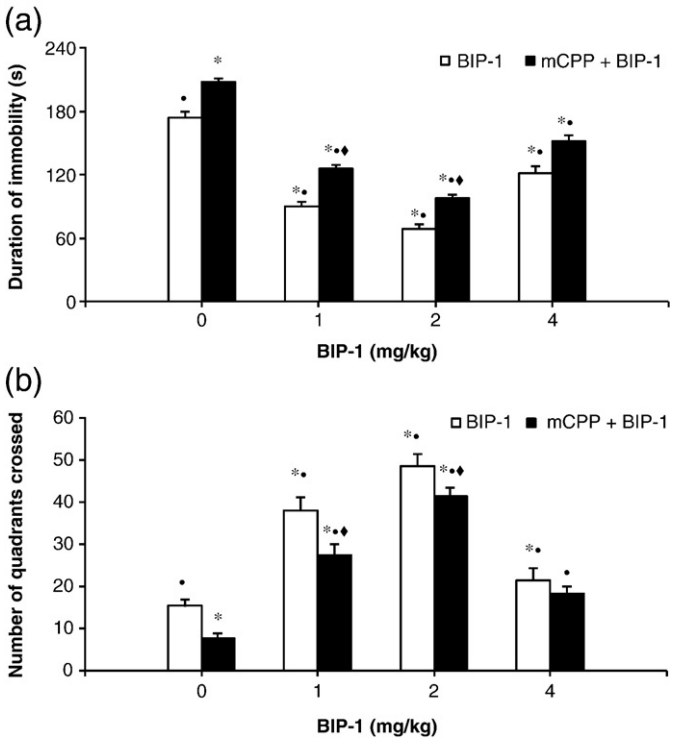

Fig. 4. Effect of mCPP (1 mg/kg) pretreatment on the antidepressant-like effects of BIP-1 in the mice FST. (a) Duration of immobility. (b) Swimming behaviour. The columns represent mean of values. Error bars indicate S.E.M. $n=8$ per group. *$P<0.05$ compared with vehicle-treated group (white column at '0' in the abscissa); •$P<0.05$ compared with mCPP alone (black column at '0' in the abscissa); ♦$P<0.05$, mCPP + BIP-1 compared with BIP-1 alone (ANOVA followed by Sidak test).

treatment, it was observed that pretreatment with mCPP attenuated the antidepressant-like effects of BIP-1 (1 and 2 mg/kg) which is evident from the increased duration of immobility (Fig. 4a) and decreased swimming behaviour (Fig. 4b). In the 8-OH-DPAT interaction study, 8-OH-DPAT and BIP-1 significantly reduced the duration of immobility ($F(7, 56)=44.19$, $P<0.05$) and increased swimming behaviour ($F(7, 56)=8.53$, $P<0.05$) compared to vehicle treatment. When comparing the effects of 8-OH-DPAT + BIP-1 combined treatment with BIP-1 treatment, it was found that pretreatment with 8-OH-DPAT weakened the antidepressant-like effects of BIP-1 as observed from increased duration of immobility and decreased swimming episodes (Fig. 5a and b).

3.4. Effects of AMI and HAR on BIP-1 pretreated mice

BIP-1 and AMI (5–20 mg/kg) exhibited a significant decrease in the duration of immobility ($F(7, 56)=76.69$, $P<0.05$) and significant increase in swimming behaviour ($F(7, 56)=11.21$, $P<0.05$) compared to vehicle treatment. BIP-1 (1 mg/kg) pretreatment augmented the decrease in the duration of immobility (Fig. 6a) induced by AMI (5–20 mg/kg) whereas, the influence on swimming behaviour was negligible (Fig. 6b). HAR (2.5–10 mg/kg) exhibited significant antidepressant-like effects and pretreatment with BIP-1 (1 mg/kg) augmented the antidepressant-like effects HAR as indicated by decreased duration of immobility ($F(7, 56)=40.30$, $P<0.05$) and increased swimming behaviour ($F(7, 56)=20.46$, $P<0.05$). The antidepressant-like effects of the BIP-1 (1 mg/kg) + HAR (10 mg/kg) combination was the most effective among all treatments (Fig 7a and b).

3.5. Effects of AMI and BIP-1 on the behaviour of OBX rats

In the modified open field exploration paradigm, OBX rats exhibited a significant increase in horizontal activity ($F(9, 50)=24.32$, $P<0.05$),

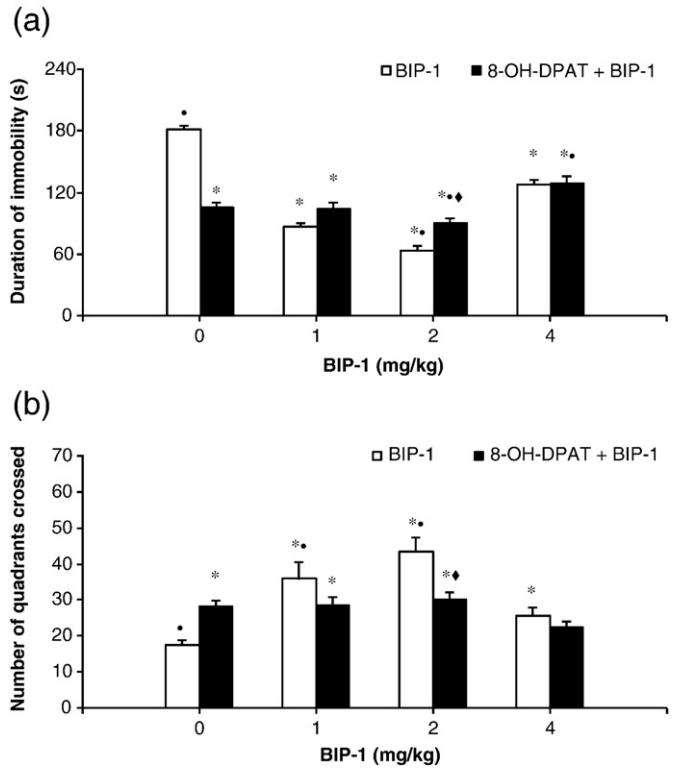

Fig. 5. Effect of 8-OH-DPAT (1 mg/kg) pretreatment on antidepressant effects of BIP-1 in the mice FST. (a) Duration of immobility and (b) swimming behaviour. The columns represent mean of values. Error bars indicate S.E.M. $n=8$ per group. *$P<0.05$ compared with vehicle-treated group (white column at '0' in the abscissa); •$P<0.05$ compared with 8-OH-DPAT alone (black column at '0' in the abscissa); ♦$P<0.05$, 8-OH-DPAT + BIP-1 compared with BIP-1 alone (ANOVA followed by Sidak test).

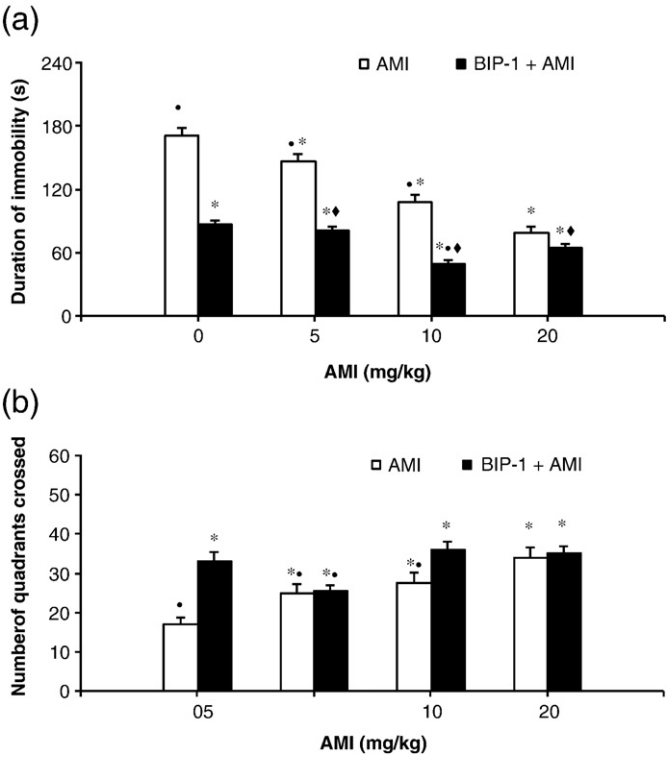

Fig. 6. Effect of BIP-1 (1 mg/kg) pretreatment on antidepressant effects of AMI in the mice FST. (a) Duration of immobility. (b) Swimming behaviour. The columns represent mean of values. Error bars indicate S.E.M. $n=8$ per group. *$P<0.05$ compared with vehicle-treated group (white column at '0' in the abscissa); •$P<0.05$ compared with BIP-1 alone (black column at '0' in the abscissa); ♦$P<0.05$, BIP-1 + AMI compared with AMI alone (ANOVA followed by Sidak test).

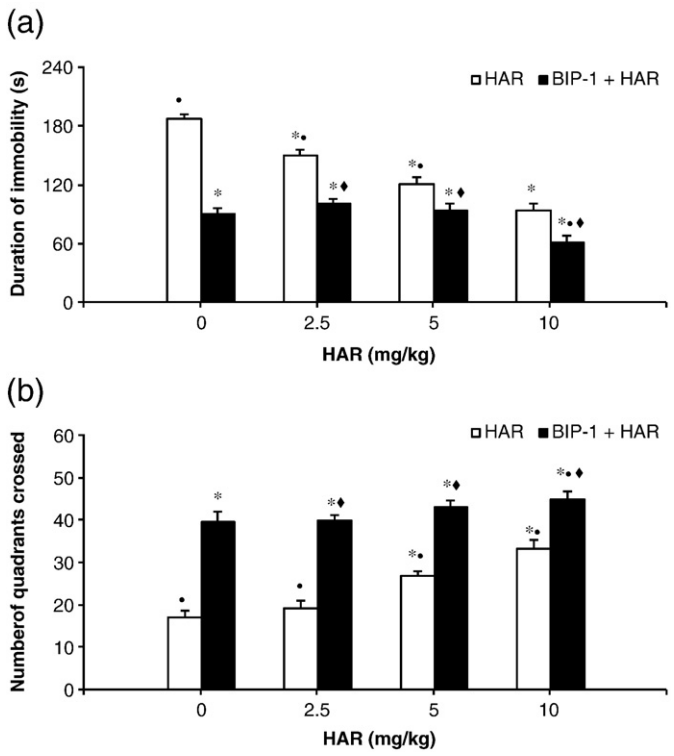

Fig. 7. Effect of BIP-1 (1 mg/kg) pretreatment on antidepressant effects of HAR in the mice FST. (a) Duration of immobility. (b) Swimming behaviour. The columns represent mean of values. Error bars indicate S.E.M. $n=8$ per group. *$P<0.05$ compared with vehicle-treated group (white column at '0' in the abscissa); •$P<0.05$ compared with BIP-1 alone (black column at '0' in the abscissa); ♦$P<0.05$, BIP-1+ HAR compared with HAR alone (ANOVA followed by Sidak test).

vertical activity ($F(7, 56)=14.41$, $P<0.05$) and defecation ($F(9, 50)=4.88$, $P<0.05$) compared to sham-operated rats. OBX rats chronically (14 days) treated with AMI (10 mg/kg) or BIP-1 (1 and 2 mg/kg) exhibited significantly reduced horizontal and vertical activities. OBX rats treated with the combination of AMI (10 mg/kg) + BIP-1 (1 mg/kg) exhibited a significant reduction in all the tested parameters when compared to vehicle, AMI (10 mg/kg) or BIP-1 (1 and 2 mg/kg) treatments. In the 7-day regimen study, except for the AMI + BIP-1 combination treatment, which significantly reduced horizontal ($F(9, 50)=13.39$, $P<0.05$) and vertical activities ($F(9, 50)=14.52$, $P<0.05$), all other treatments failed to show any significant change in the tested parameters (Table 3). OBX rats spent less time in active interaction and exhibited a greater number of crossings compared to sham-operated rats in the social interaction paradigm. Chronic treatment with AMI (10 mg/kg), BIP (1 and 2 mg/kg) or combination of AMI (10 mg/kg) + BIP-1 (1 mg/kg) significantly increased the interaction time ($F(9, 50)=12.69$, $P<0.05$) and decreased the number of crossings ($F(9, 50)=10.85$, $P<0.05$) compared to vehicle treatment (Table 4). The combination of AMI (10 mg/kg) + BIP-1(1 mg/kg) was the most effective among all treatments in reversing the OBX induced behaviour. In the 7-day regimen study, the combination treatment significantly increased the interaction time ($F(9, 50)=19.18$, $P<0.05$) and decreased the crossing ($F(9, 50)=11.31$, $P<0.05$) compared to vehicle treatment (Table 4).

OBX rats showed an enhanced emotional behaviour compared to sham-operated rats (Fig. 8a and b). Such an effect was significantly ($F(9, 50)=34.19$, $P<0.05$) reduced by chronic AMI (10 mg/kg), BIP-1 (1 and 2 mg/kg) or the combination of AMI (10 mg/kg) + BIP (1 mg/kg) treatment, when compared to vehicle treatment. When observing the data from 7-day regimen study it is evident that among the various treatments, only the combination of AMI (10 mg/kg) + BIP-1 (1 mg/kg) significantly ($F(9, 50)=32.32$, $P<0.05$) reduced the total hyperemotionality score compared to vehicle treatment (Fig. 8a). OBX rats exhibited a significanlty decreased sucrose consumption when compared to sham-operated rats (Fig. 9a). All the chronic drug treatments significantly increased sucrose consumption in OBX rats ($F(9, 50)=9.41$, $P<0.05$) when compared with the vehicle treatment (Fig. 9a). In the 7-day regimen study, the sucrose consumption was significantly ($F(9, 50)=4.69$, $P<0.05$) reduced in OBX rats on the 7th day. Although an antidepressant-like trend was evident, the increase in sucrose consumption observed in the BIP-1 or AMI treated groups failed to attain the level of statistical significance (Fig. 9b). The drug treatments did not notably influence the behaviour of sham-operated animals in any of the paradigms mentioned above.

4. Discussion

The results of this behavioural investigation divulge the antidepressant-like effects of BIP-1. The preliminary attributes of BIP-1 namely (i) Log *P* value of 2.11, which is optimum for blood brain barrier permeability (Ter Laak et al., 1994), (ii) 5-HT_{2A} receptor affinity inferred from the receptor binding assay and (iii) antidepressant-like effects observed in mice FST, incentivised the present study which comprised a series of standardised antidepressant assays. The central nervous system (CNS) stimulatory or sedative property of a test compound mimics antidepressant-like or depressogenic-like behavioural outcome of rodents respectively, in forced swim test (Porsolt et al., 1978). BIP-1 (0.5–2 mg/kg) exhibited significant antidepressant-like effects in FST as well as TST (a subtly variant version of FST) and the tested dose range did not alter the basal SLA.

Table 3
Effects of AMI, BIP-1 and the combination treatment on the behaviour of OBX and sham-operated rats in modified open field exploration paradigm.

Treatment	Horizontal activity		Vertical activity		Defecation	
	7th day	14th day	7th day	14th day	7th day	14th day
OBX rats						
Vehicle	178.50 ± 12.50♦	219.00 ± 17.03♦	21.83 ± 1.30♦	24.17 ± 2.12♦	4.67 ± 0.93♦	5.17 ± 1.45♦
AMI (10 mg/kg)	168.67 ± 7.74	125.50 ± 8.80[a]	20.27 ± 2.50	11.83 ± 1.70[a]	4.50 ± 0.43	2.33 ± 0.49
BIP-1 (1 mg/kg)	170.00 ± 11.00	135.33 ± 14.19[a]	18.67 ± 2.53	13.67 ± 2.32[a]	4.67 ± 0.49	3.67 ± 0.88
BIP-1 (2 mg/kg)	163.89 ± 6.47	128.67 ± 9.91[a]	19.33 ± 2.29	7.33 ± 1.14[a]	4.33 ± 0.76	2.00 ± 0.59
AMI (10 mg/kg) + BIP-1 (1 mg/kg)	141.67 ± 7.74[a,b,c,d]	103.33 ± 12.7474[a,b,c,d]	11.67 ± 1.87[a,c]	5.33 ± 1.15[a,c]	2.50 ± 0.43	1.00 ± 0.37[a]
Sham-operated rats						
Vehicle	95.83 ± 4.72	91.17 ± 6.86	7.00 ± 0.77	6.83 ± 0.60	2.50 ± 0.43	2.17 ± 0.48
AMI (10 mg/kg)	92.67 ± 6.59	94.67 ± 6.14	7.83 ± 0.79	8.67 ± 0.71	1.83 ± 0.48	2.00 ± 0.58
BIP-1 (1 mg/kg)	98.67 ± 6.93	94.17 ± 9.27	7.88 ± 0.66	7.50 ± 0.67	2.33 ± 0.43	2.33 ± 0.71
BIP-1 (2 mg/kg)	95.83 ± 5.90	93.00 ± 7.61	8.50 ± 0.81	8.33 ± 1.15	2.17 ± 0.43	2.00 ± 0.86
AMI (10 mg/kg) + BIP-1 (1 mg/kg)	97.17 ± 6.72	95.80 ± 10.03	8.00 ± 0.73	8.35 ± 1.23	2.30 ± 0.56	2.17 ± 0.87

Values are mean ± S.E.M. $n=6$ per group. [a]$P<0.05$ compared with vehicle-treated OBX rats. [b]$P<0.05$ compared with AMI treated OBX rats. [c]$P<0.05$ compared with BIP-1 (1 mg/kg) treated OBX rats. [d]$P<0.05$ compared with BIP-1 (2 mg/kg) treated OBX rats. ♦$P<0.05$ compared with vehicle-treated sham rats (ANOVA followed by Sidak test).

Table 4
Effects of AMI, BIP-1 and the combination treatment on the behaviour of OBX and sham-operated rats in the social interaction paradigm.

Treatment	Interaction time		Number of crossing	
	7th day	14th day	7th day	14th day
OBX rats				
Vehicle	24.83 ± 2.40♦	15.00 ± 1.71♦	22.50 ± 1.75♦	27.30 ± 1.67♦
AMI (10 mg/kg)	28.33 ± 1.92	42.33 ± 7.03[a]	19.67 ± 1.84	16.33 ± 1.45[a]
BIP-1 (1 mg/kg)	26.07 ± 2.63	36.00 ± 3.83[a]	19.33 ± 2.29	18.41 ± 1.29[a]
BIP-1 (2 mg/kg)	29.33 ± 4.44	51.33 ± 6.07[a]	18.00 ± 1.69	13.33 ± 1.61[a]
AMI (10 mg/kg) + BIP-1 (1 mg/kg)	37.83 ± 3.24[a,b,c]	62.50 ± 9.96[a,b,c]	11.50 ± 1.84[a]	10.50 ± 1.18[a,c]
Sham-operated rats				
Vehicle	59.83 ± 6.85	57.33 ± 6.76	10.67 ± 1.38	10.33 ± 1.28
AMI (10 mg/kg)	56.67 ± 5.95	53.67 ± 5.20	10.17 ± 0.99	9.67 ± 1.26
BIP-1 (1 mg/kg)	54.83 ± 6.38	52.67 ± 5.73	9.50 ± 1.06	9.50 ± 1.06
BIP-1 (2 mg/kg)	56.67 ± 5.38	52.83 ± 7.70	10.33 ± 1.15	10.17 ± 1.42
AMI (10 mg/kg) + BIP-1 (1 mg/kg)	54.5o ± 3.25	52.00 ± 5.45	9.67 ± 0.88	10.00 ± 1.39

Values are mean ± S.E.M. $n = 6$ per group. [a]$P < 0.05$ compared with vehicle-treated OBX rats; [b]$P < 0.05$ compared with AMI treated OBX rats; [c]$P < 0.05$ compared with BIP-1 (1 mg/kg) treated OBX rats; ♦$P < 0.05$ compared with vehicle-treated sham rats (ANOVA followed by Sidak test).

Hence the behaviour of mice in the above mentioned assays does not reflect a sheer CNS stimulatory effect of BIP-1. At the dose level of 4 mg/kg, BIP-1 failed to significantly increase the swimming behaviour (in mice FST), which is correlated with the decreased SLA observed at the same dose level. This finding is in consonance with earlier studies that reported sedative properties of 5-HT_{2A} receptor antagonists in rodents (Redrobe and Bourin, 1997; Olivier et al., 1998; Borsini et al., 2002).

Quipazine induces a characteristic head twitch response in rodents by stimulating the postsynaptic 5-HT_{2A} receptors (Malick et al., 1977; Green et al., 1983; Schreiber et al., 1995; Nacca et al., 1998). The reversal of quipazine induced head twitches by BIP-1 (0.25–4 mg/kg) treatment and the high 5-HT_{2A} receptor affinity of BIP-1 inferred from receptor binding assay (unpublished results), substantiates the involvement of 5-HT_{2A} receptors in the antidepressant-like effects of BIP-1. The biphasic dose–response relationship noted in the quipazine induced HTR study, FST and TST can be reasoned as follows. BIP-1, which was designed as an atypical antipsychotic has affinity to both 5-HT_{2A} and D_2 receptors. Stimulation of D_2 receptors instigates an antidepressant-like behavioural outcome in rodents (Willner, 1997;

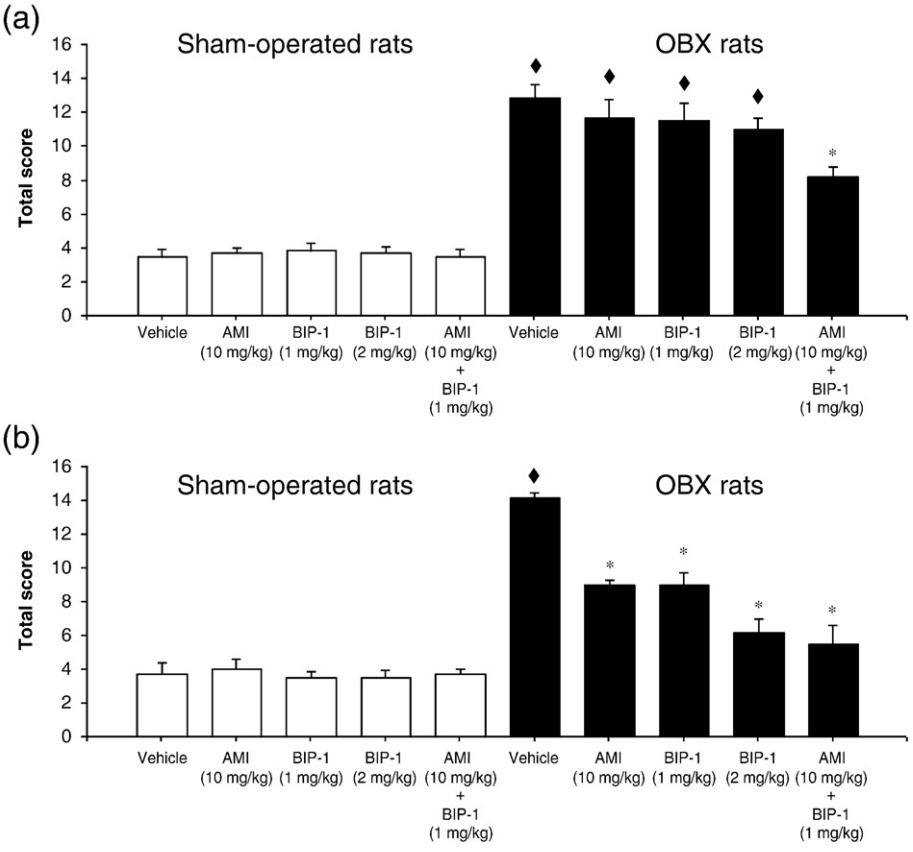

Fig. 8. Effects of AMI, BIP-1 and combination treatment on hyperemotionality scores of OBX and sham-operated rats. (a) 7th day scores. (b) 14th day scores. Columns represent mean of total emotionality scores and error bars indicate S.E.M. $n = 6$ per group. *$P < 0.05$ compared with vehicle-treated OBX rats; ♦$P < 0.05$ compared with vehicle-treated sham-operated rats (ANOVA followed by Sidak test).

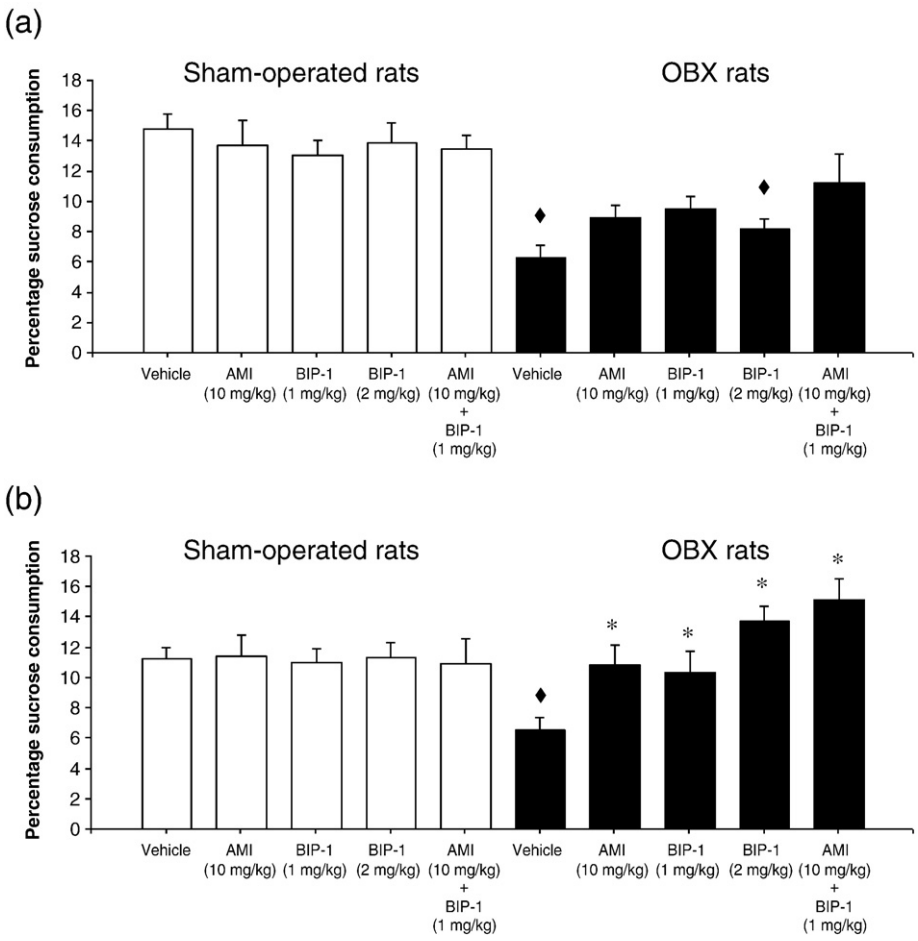

Fig. 9. Effects of AMI, BIP-1 and combination treatment on sucrose consumption of OBX and sham-operated rats. (a) 7th day recording. (b) 14th day recording. Columns represent mean of percentage sucrose consumption and error bars indicate S.E.M. $n = 6$ per group. $^{*}P < 0.05$ compared with vehicle-treated OBX rats; $^{\blacklozenge}P < 0.05$ compared with vehicle-treated Sham-operated rats (ANOVA followed by Sidak test).

Siuciak and Fujiwara, 2004; Basso et al., 2005; Brocco et al., 2006). Therefore, at the higher dose level BIP-1 is likely to antagonise D_2 receptors, which eventually diminishes the antidepressant-like effect. Nevertheless, further interaction studies of BIP-1 with selective dompaminergic ligands are obligatory to confirm the dopaminergic involvement.

mCPP is a nonselective 5-HT_2 receptor agonist, which is classified as a neuroendocrine probe to assess the serotonergic function (Yatham and Steiner, 1993; Anand et al., 1994). Previous studies in our laboratory have shown that acute administration of mCPP (1 mg/kg) induces a depressogenic-like effects in rodents, and this effect is chiefly mediated by stimulation of 5-HT_{2A} receptors (Mahesh et al., 2007; Rajkumar et al., 2009). The present data reveal that BIP-1 significantly attenuated the depressogenic-like effects of mCPP, which further supports the involvement of 5-HT_{2A} receptors. 8-OH-DPAT (a selective 5-HT_{1A} receptor agonist) exhibits antidepressant-like effects in mice FST (Luscombe et al., 1993; O'Neill and Conway, 2001; Ramamoorthy et al., 2008), and such an effect can be potentiated by 5-HT_{2A} antagonists (Celada et al, 2004; Stahl, 2008). In this study, 8-OH-DPAT pretreatment did not potentiate the antidepressant-like effects of BIP-1 (on the contrary supressed the effects) which implies the involvement of other receptor mechanisms. 5-HT_{2A} receptors are partly involved in the antidepressant-like effects of TCAs in mice FST (Redrobe and Bourin, 1997). BIP-1 augmented the antidepressant-like effects of AMI (a TCA), and such an effect was also evident even in OBX rats (discussed below). HAR, a β-carboline alkaloid, increases the monoamine levels not only by inhibiting the enzyme monaoamine oxidase-A and B (May et al., 1991; Rommelspacher et al., 1994; Farzin and Mansouri, 2006) but also by inhibiting serotonin reuptake (Sällström-Baum et al., 1996). Pretreatment with BIP-1 (1 mg/kg) enhanced the antidepressant-like effects of HAR indicating the facilitatory effects of BIP-1 on the serotonergic neurotransmission. In short, the interaction study with various ligands points to the 5-HT_{2A} receptor mediated mechanism behind the antidepressant-like effects of BIP-1.

Unlike other rodent models, the OBX represents a model of chronic agitated hyposerotonergic depression (Lumia et al., 1992) which is sensitive to detect the antidepressant-like effects of agents affecting the 5-HT receptor subtypes (for reviews see Kelly et al., 1997; Song and Leonard, 2005). Furthermore, it is reported that the antidepressant-like effects of test compounds are noticeable only after chronic treatment in OBX rats(van Riezen and Leonard, 1990; Cryan et al., 1998; Kelly et al., 1997; Mahesh et al., 2007). This resembles the requirement of chronic drug treatment for improvement in clinical signs of human depressive disorder. In the present study, OBX rats displayed a wide range of behavioural abnormalities, such as (i) increased horizontal and vertical activities and defecation in modified open field exploration paradigm, (ii) decreased active interaction and increased crossing in social interaction paradigm and (iii) increased hyperemotionality scores as reported earlier (Kelly et al., 1997, Harkin et al., 2003, Ramamoorthy et al., 2008; Pandey et al., 2008). Furthermore, the decreased sucrose consumption observed in the OBX rats is a measure of anhedonia, a characteristic feature of endogenous depression (Willner et al., 1987; Willner, 1990; Muscat et al., 1990; Monleon et al., 1995; Wang et al., 2009). The chronic regimen of AMI significanlty reversed the bulbectomy induced behaviour in all the

aforementioned paradigms. This result is in line with previous reports on reversal of bulbectomy induced behaviour by TCAs (Nurimoto et al., 1974; Shibata et al., 1984; O'Connor and Leonard, 1988; van Riezen and Leonard 1990; Mar et al., 2000; Xu et al., 2005). Another notable similarity between human depression and OBX rat is the altered 5-HT_{2A} receptor binding and function (Butler and Leonard, 1988; Arango et al., 1990; Gurevich et al., 1993; Earley et al., 1994; Mudunkotuwa and Horton, 1996; Grecksch et al., 1997; Massou, 1997; Messa, 2003). Trazodone restored the avoidance learning in bulbectomised c57 mice and this effect was correlated with down-regulation of 5-HT_2 receptors in the frontal cortex (Gurevich et al., 1993). Together with the data from the interaction studies in mice, the reversal of bulbectomy induced behavioural changes caused by BIP-1 treatment, corroborates the role of 5-HT_{2A} receptor in the antidepressant-like effects of BIP-1.

Early-onset of action is the prime requirement of antidepressant drug treatment (Derivan, 1995; Montgomery, 1997; Gelenberg and Chesen, 2000; Artigas, 2001) and several strategies have been proposed to achieve the same (Dremencov et al., 2004; Machado-Vieira et al., 2008). It is a noteworthy finding that the antidepressant-like effects of the combination of BIP-1 and AMI were evident with a short course of treatment (7 days) in OBX rats. BIP-1 treatment can plausibly lead to a functional blockade of 5-HT_{2A} receptors which eventually results in enhanced 5-HT and norepinephrine neurotransmission (Gobert and Millan, 1999; Blier, 2003). The synaptic norepinephrine levels are further increased when AMI is co-administered with BIP-1, due to the inhibiton of norepinephrine reuptake by AMI. Hence, an early-onset of antidepressant-like effect is feasible due to the involvement of both noradrenergic and serotonergic neurotransmission systems (Blier 2003). Moreover, based on clinical observations it is noted that 5-HT_{2A} receptor antagonism is a candidate mechanism for effective and early-onset antidepressant action (Borsini et al., 1997; Marek et al., 2003, Dremencov et al., 2004; Adell et al., 2005; Rasmussen, 2006). Thus, we conclude that BIP-1 exhibits antidepressant-like effects in this test battery and cogent evidence suggests that antagonism of 5-HT_{2A} receptors is the cardinal underlying mechanism. Coadministration of TCAs with the molecules of this class (following the establishment of safety profile), can be contemplated as an approach to achieve an early-onset of antidepressant action in humans.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.pbb.2009.09.018.

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