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EFFECT OF FLUVOXAMINE AND N-METHYL-D-ASPARTATE RECEPTOR ANTAGONISTS ON SHOCK-INDUCED DEPRESSION IN MICE

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Abstract : We have earlier demonstrated that NMDA receptor antagonists possess antidepressant effect and also they show a synergism with imipramine. The present study attempts to investigate whether NMDA receptor antagonists also interact with selective serotonin reuptake inhibitors. The study was conducted in albino mice using shock-induced depression model. The mice were placed on a grid floor and shock delivered were of 2 sec duration with a 9 sec interval for 1h. Twenty four hours later depression was measured by an open field test followed by a forced swimming test. Presentation of inescapable foot shock significantly reduced ambulation (from 159.50 ± 5.42 to 80.50 ± 4.61) and rearing (from 22.10 ± 2.15 to 11.30 ± 1.32) in the open field arena and increased immobility duration in the forced swimming test (from 82.20 ± 3.51 to 158.90 ± 4.61). Pretreatment with fluvoxamine, MK-801, ketamine and the combination of fluvoxamine with either of the NMDA antagonists antagonised shock-induced depression. Haloperidol and ketanserin pretreatment modified the effect of these agents. These findings suggest an interaction of NMDA receptor antagonists with fluvoxamine, and an involvement of brain dopaminergic and tryptaminergic mechanisms in the behavioural suppression observed after inescapable foot shock.

Key words : fluvoxamine NMDA receptors MK–801 ketamine shock-induced depression

INTRODUCTION

Over-activation of N-methyl-D-aspartate (NMDA) receptors has been suggested to be responsible for several human neuropathologies. The development of compounds that block these receptors has given impetus to research in this field. The high affinity of the NMDA antagonists for their receptors coupled with rapid CNS penetration, long duration of action and uncompetetive antagonism makes these compounds attractive for clinical use (1,2). Antagonists of NMDA receptors have been

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demonstrated to have anxiolytic, anticonvulsant, muscle relaxant and neuroprotective actions in various preclinical models (1).

In our earlier work, it was demonstrated that NMDA receptor antagonists reduce depression in mice induced by inescapable shock, and that imipramine has synergistic effect (2). Similar findings were observed in forced swimming test (FST) paradigm (3). The present study was designed to investigate whether fluvoxamine, a selective serotonin reuptake inhibitor (SSRI), shows a similar synergism with NMDA receptor antagonist in shock-induced depression model.

METHODS

Animals

Adult albino mice of either sex, weighing 25 ± 5g, raised in the Central Animal Facility of Maulana Azad Medical College, were used. They were maintained at a 12h day/night cycle, and were acclimatized to the laboratory conditions 24h before testing and had free access to food and water. The study was conducted between 0800 and 1200 hours.

Drugs

The drugs used in the present study were fluvoxamine maleate (Kali-Duphar Pharma.), MK-801 (Merck Research Laboratories), ketamine hydrochloride (Themis Chemicals Ltd.), haloperidol (Searle India Ltd.), and ketanserin tartarate (Janssen Pharmaceutica). All the drug solutions were freshly prepared in saline and were injected intraperitoneally in a fixed volume of 5 ml/kg.

While fluvoxamine, MK-801 and ketamine were administered 30 min before; haloperidol and ketanserin were administered 60 min before the shock session. Control animals received 0.9% saline in similar volume.

Shock-induced depression

The method adopted was same as used earlier by Chaturvedi *et al* (2).

(i) Delivery of Shock: Four mice were placed on a grid floor (26 × 26 cm) made of stainless steel rods (2 mm diameter, placed 1cm apart) connected in series. The animals were prevented from escaping or coming in contact with each other by inverting separate glass beakers over them. The electric schock generator (Medicare Research Stimulator SB44, Recorders & Medicare Systems, India) was programmed to deliver 300μA foot shocks of 2 sec duration at intervals of 9 sec. The animals were shocked for a total of 1h. Special attention was paid to keep the grid clean from faecal matter to avoid short circuits terminating the shock delivery. Control animals were only placed on the grid under inverted beakers for 1h but were not shocked.

(ii) Behavioural Testing: Twenty-four hours after the shock administration,

behavioural depression was measured by an open field test (OFT) followed by a forced swimming test (FST).

a. The OFT was carried out in a circular wooden arena (84 cm diameter, 30 cm high) with a white sunmica base with three concentric circles divided into segments by radial lines originating from the centre. Each animal was tested for 5 min. Ambulation (locomotor behaviour) was measured as number of lines crossed by an animal, and rearing (exploratory activity) was measured as number of times the animal stood on its hind limbs with or without the support of circular wall. The counting of ambulation and rearing responses was done using a hand operated counter.

b. Immediately after the behaviour testing procedure the animals were subjected to FST. The animals were forced to swim individually, for 6 min, in a glass beaker (11 cm diameter, 15 cm high) containing fresh water upto a height of 6 cm and maintained at a temperature of 22 ± 1°C. Each animal made vigorous attempts to get out of glass beaker during the first few minutes and thereafter became immobile with occasional escape attempts. The total duration of immobility during the last 4 min of the 6 min test period was recorded.

Statistcal analysis

Statistical analysis of data was performed using Student's t-test, one way analysis of variance (ANOVA) followed by either Dunnet's test or Tukey's multiple range test, wherever appropriate.

RESULTS

Mice exposed to inescapable foot shock for 1 h had reduced activity in both OFT and FST as compared to controls (Table I).

TABLE I: Effect of inescapable foot shock on behaviour in mice (mean ± SEM; n = 10).

	OFT		*FST*
	Ambulation	*Rearing*	*Immobility*
Control	159.50±5.42	22.10±2.15	82.20±3.51
Shocked	80.50±4.61**	11.30±1.32**	158.90±4.61**

OFT = open test, FST = forced swimming test. The OFT values represent number of episodes in 5 min and the FST values denote the duration of immobility in last 4 min of a 6 min test. **P<0.001 Vs Control using Student's t-test.

TABLE II : Effect of fluvoxamine, MK 801 and ketamine on shock-induced changes in behaviour in mice (mean ± SEM; n = 10).

Treatment (mg/kg)	*OFT*		*FST*
	Ambulation	*Rearing*	*Immobility*
Control	88.62±4.69	11.25±1.21	159.12±6.16
Fluvoxamine (5)	96.85±4.00	12.14±1.31	149.42±4.67
Fluvoxamine (10)	95.62±5.92	16.12±1.65*	123.37±4.30**
Fluvoxamine (20)	98.00±7.18	20.87±2.90**	95.50±5.21**
Control	78.62±4.98	10.25±2.33	155.00±5.45
MK-801 (0.05)	94.12±6.46	13.00±1.08	148.00±4.73
MK-801 (0.1)	112.62±4.67**	18.25±1.54**	122.25±5.59**
MK-801 (0.2)	144.87±6.04**	27.12±1.93**	77.00±4.79**
Control	81.70±5.61	10.10±1.75	155.20±5.37
Ketamine (2.5)	86.37±5.15	14.37±1.52	150.87±5.22
Ketamine (5)	100.25±4.88*	20.75±2.01**	132.62±4.58*
Ketamine (10)	124.00±6.74**	29.25±2.44**	102.62±4.16**

OFT = open field test, FST = forced swimming test. The OFT values represent number of episodes in 5 min and the FST values denote duration of immobility in last 4 min of a 6 min test. *P<0.05 and **P<0.01 Vs Control using ANOVA and Dunnett's test.

Pretreatment with fluvoxamine (5, 10 and 20 mg/kg, MK 801 (0.05, 0.1 and 0.2 mg/kg) and ketamine (2.5, 5 and 10 mg/kg) significantly reversed this effect of shock on behaviour. The only difference between fluvoxamine and MK-801 or ketamine was that fluvoxamine failed to antagonise the effect of foot shock on ambulatory behaviour (Table II).

Haloperidol (0.1 mg/kg) *per se* neither did modify the effect shock, nor the effect of fluvoxamine was altered in its presence. The effect of both MK 801 and ketamine was antagonised by haloperidol pretreatment. The effect of fluvoxamine with either of the NMDA antagonist on rearing and immobility duration were attenuated by haloperidol, whereas the effect of the combinations on ambulation was antagonised (Table III).

Table IV gives the modification of the effect of fluvoxamine and NMDA antagonists by ketanserin (4 mg/kg). Though, ketanserin

TABLE III : Modification of the effect of fluvoxamine, MK-801 and ketamine by haloperidol on shock-induced changes in behaviour in mice (mean ± SEM; n = 8–10).

Treatment (mg/kg)	*OFT*		*FST*
	Ambulation	*Rearing*	*Immobility*
Control	91.50±4.68	11.25±1.22	156.87±4.29
Haloperidol (0.1)	89.37±6.11	10.00±1.87	160.75±7.44
Fluvoxamine (10)	95.75±6.75	21.00±1.75**	121.50±5.81**
Haloperidol (0.1) + Fluvoxamine (10)	90.87±7.44	22.50±1.19**	129.00±4.72**
MK-801 (0.1)	115.37±5.79*	20.75±1.42**	118.50±5.05**
Haloperidol (0.1) + MK-801 (0.1)	90.87±7.44[b]	9.37±1.43[b]	150.87±7.73[b]
Ketamine (5)	105.50±4.76**	18.75±1.80*	129.87±3.16**
Haloperidol (0.1) + Ketamine (5)	88.75±6.59[d]	11.50±1.26[d]	157.75±7.61[d]
Fluvoxamine (10) + MK-801 (0.1)	135.50±4.12**[ab]	30.63±1.66**[ab]	95.25±5.30**[ab]
Fluvoxamine (10) + Ketamine (5)	130.13±4.07**[ad]	28.40±2.09**[ad]	95.50±4.50**[ad]
Haloperidol (0.1) + Fluvoxamine (10) + MK-801 (0.1)	98.62±5.57[c]	20.12±1.66**[c]	125.25±4.86**[c]
Haloperidol (0.1) + Fluvoxamine (10) + Ketamine (5)	99.50±7.75[e]	20.80±1.49**[e]	128.12±6.41**[e]

OFT = open field test, FST = forced swimming test. The OFT values represent number of episodes in 5 min and the FST values denote duration of immobility in last 4 min of a 6 min test. *P<0.05 and **P<0.01 Vs Control using ANOVA and Dunnett's test. Other comparisons: [a]Vs Fluvoxamine (10); [b]Vs MK-801 (0.1); [c]Vs Fluvoxamine (10) + MK 801 (0.1); [d]Vs Ketamine (5) and [e]Vs Fluvoxamine (10) + Ketamine (5) using ANOVA and Tukey's multiple range test at 5% level.

per se failed to modify the effect of inescapable foot shock, it antagonised the effect of fluvoxamine on immobility duration. Ketanserin failed to modify the effect of either of the NMDA antagonists. The effect of combination of fluvoxamine with both, MK 801 and ketamine, on immobility duration was attenuated by ketanserin pretreatment.

TABLE IV: Modification of the effect of fluvoxamine, MK 801 and ketamine by ketanserin on shock-induced changes in behaviour in mice (mean ± SEM; n = 8–10).

Treatment (mg/kg)	*OFT*		*FST*
	Ambulation	*Rearing*	*Immobility*
Control	86.87±6.51	10.50±1.26	154.37±5.21
Ketanserin (4)	89.22±5.03	10.44±1.41	154.25±6.31
Fluvoxamine (10)	95.62±5.91	19.25±1.59*	123.37±4.30**
Ketanserin (4) + Fluvoxamine (10)	96.00±7.72	18.71±1.96*	154.14±7.02[a]
MK-801 (0.1)	116.40±5.77**	20.63±1.53**	122.25±5.59**
Ketanserin (4) + MK-801 (0.1)	117.12±6.85**	20.25±2.22**	120.75±7.57**
Ketamine (5)	109.75±4.99**	19.81±1.67*	126.50±5.80**
Ketanserin (4) + Ketamine (5)	112.00±6.44**	18.87±2.93*	119.62±5.55**
Fluvoxamine (10) + MK-801 (0.1)	139.50±6.57**[ab]	31.13±2.64**[ab]	92.88±4.37**[ab]
Fluvoxamine (10) + Ketamine (5)	142.62±5.11**[ad]	31.00±1.91**[ad]	98.37±5.35**[ad]
Ketanserin (4) + Fluvoxamine (10) + MK-801 (0.1)	141.00±6.14**	29.75±2.63**	124.62±5.81**[e]
Ketanserin (4) + Fluvoxamine (10) + Ketamine (5)	140.10±4.50**	28.80±2.42**	129.13±6.68**[e]

OFT = open field test, FST = forced swimming test. The OFT values represent number of episodes in 5 min and the FST values denote duration of immobility in last 4 min of a 6 min test. *P<0.05 and **P<0.01 Vs Control using ANOVA and Dunnett's test. Other comparisons: [a]Vs Fluvoxamine (10); [b]Vs MK-801 (0.1); [c]Vs Fluvoxamine (10) + MK 801 (0.1); [d]Vs Ketamine (5) and [e]Vs Fluvoxamine (10) + Ketamine (5) using ANOVA and Tukey's multiple range test at 5% level.

DISCUSSION

Acute uncontrollable stressors have been shown to increase the utilization of catecholamines and serotonin (5-HT), leading to reduced levels of these monoamines in various regions of the brain (4, 5). Behavioural depression following an acute stressor might result from tryptaminergic mechanisms or may be attributed to a motor activation deficit stemming from reduction of norepinephrine (NE) (6, 7). We have earlier demonstrated that exposure to inescapable foot shock leads to behavioural alteration in mice (2). In the present study also, exposure to inescapable foot shock led to a decrease of both ambulation and rearing behaviour of mice in the OFT and increased immobility duration in the FST.

Fluvoxamine could only party antagonise the effect of inescapable foot shock. It is well established that uncontrollable stressors deplete NE and dopamine (DA) besides 5-HT (8), and both NE and DA are considered to be important for locomotor activity (9). Therefore, it seems that partial effect of fluvoxamine in the present study is a consequence of its 5-HT selectivity. Our finding is in agreement with those of other workers (10, 11). Furthermore, Plaznik *et al* have shown that various tryptaminergic agents including citalopram failed to improve the deficient open field behaviour 24h after foot shock treatment (12).

The immobility reducing effect of fluvoxamine was antagonised by ketanserin pretreatment. This supports the role of tryptaminergic mechanisms. Though, fluvoxamine could also increase rearing activity, this effects was not antogonised by either haloperidol, or by any other antagonist used. Dandiya *et al* described rearing activity in rodents as a complex pattern of stereotyped behaviour (13). As the increased rearing due to fluvoxamine was not antagonised by haloperidol it can not be explained in terms of brain DA levels. It seems probable that the increased rearing with fluvoxamine is a consequence of CNS arousal. Indeed, Gupta et al have demonstrated that CNS arousal facilitates rearing (14).

In the present work, both NMDA receptor antagonists, MK-801 and ketamine antagonised the effect of inescapable foot shock in a dose dependent fashion. The increased ambulation and rearing in the OFT and decreased duration of immobility in the FST due to these agents was antagonized by haloperidol pretreatment. Ketanserin failed to modify these effects. These results are in agreement with those of Meloni *et al* who have shown that dizocilpine (MK-801) diminishes the behavioural deficit produced by learned helplessness (15). An indirect DA activation, through blockade of NMDA receptors, has been proposed for MK-801 by a number of workers (16–18). Recently, it has been demonstrated that haloperidol acts as a selective inhibitor of MK-801 binding to the NMDA receptors (19). Irifune *et al* have also demonstrated that ketamine induced locomotion is antagonized by haloperidol, in a dose which does not affect spontaneous locomotor activity (20). Moreover, the destruction of dopaminergic neurons resulted in the suppression of ketamine-induced locomotor activity, suggesting that the presence of intact dopaminergic neurons was indispensable for the response of ketamine.

The concomitant administration of either MK-801 or ketamine with fluvoxamine increased the ambulatory activity of mice in the OFT. Similar observation was made by Maj *et al* when MK-801 was administered with fluoxetine or citalopram, both selective 5HT uptake inhibitors (21, 22). In our study this effect was antagonised by haloperidol pretreatment. This probably is a consequence of DA release facilitating effect via tryptaminergic activation, as has been shown by Benloucif and Galloway (23). It thus, suggests a link between tryptaminergic and dopaminergic systems. The increased rearing activity observed with concomitant administration of fluvoxamine with either of the NMDA antagonists was partly antagonised by pretreatment

with holoperidol. Ketanserin could modify this behaviour. Though, dopaminergic mechanism is involved in rearing, the incomplete antagonism by haloperidol suggests that other non-dopaminergic mechanisms exists. The effect of these combinations on the duration of immobility in the FST after inescapable foot shock was attenuated by both ketanserin and holoperidol. This suggests a role of both dopaminergic and tryptaminergic neurotransmission in the FST.

An analysis of the results of this study revealed an antidepressant profile of the NMDA receptor antagonists, and a complexity of neurotransmitter mechanisms responsible for the occurrence of behavioural effects in this model. The present study demonstrated that brain dopaminergic and tryptaminergic mechanisms are involved in the behavioural suppression observed after inescapable foot shock and both fluvoxamine and the NMDA receptor antagonists are capable of antagonising the effects of inescapable foot shock.

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