SUMMARY AND CONCLUSIONS

CHAPTER 1: RELATIVE SENSITIVITY OF ANTIDEPRESSANT DRUGS IN BEHAVIORAL PARADIGMS OF DESPAIR

The forced swim test (FST) and tail suspension test (TST) are widely used as animal models for screening potential antidepressants. Immobility or despair behavior (helplessness) produced in both FST and TST are taken as paradigm of depression and this behavior is sensitive to reversal by antidepressant drugs. There have been some recent studies which have shown hemodynamic, behavioural, physiological and pharmacological variations in these two models, besides the influence of strain of animals used in testing in these test models. The present study was undertaken to compare the antidepressant profiles of four major classes of antidepressants namely tricyclics (imipramine), selective serotonin reuptake inhibitor (fluoxetine), dual reuptake inhibitor of serotonin and norepinephrine (venlafaxine) and atypical antidepressants (mianserin and trazodone) in male laca strain of mice subjected to two test procedures, namely FST and TST. Total immobility period was recorded for a period of six minutes in both the tests and the results were expressed as percentage change (decrease) in immobility period with respect to vehicle control. Chlorpromazine (4 mg/kg., i.p.) or pentobarbitone (20 mg/kg., i.p.) were used as negative control. Imipramine (2, 5, 10 and 20 mg/kg), fluoxetine (5, 10, 20 and 40 mg/kg), or venlafaxine (2, 4, 8 and 16 mg/kg) dose dependently decreased the immobility period. ED$_{50}$ values of imipramine, fluoxetine, and venlafaxine in FST and TST were found to be 9.2, 10 mg/kg i.p, 18, 20 mg/kg., i.p., and 8.5, 12 mg/kg., i.p, respectively. The relative potency order of these standard drugs in both FST and TST was imipramine=venlafaxine>fluoxetine. Mianserin (16 and 32 mg/kg., i.p.) or trazodone (1 and 2 mg/kg., i.p.), the two atypical antidepressants were ineffective to reduce the immobility period in both the tests. Chlorpromazine or pentobarbitone did not affect the immobility period thus
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showing that the test procedure is sensitive to antidepressants only. The present study further validated that both the test procedures are equi-sensitive to antidepressant drugs of different class in the strain of animals used.

CHAPTER 2: ANTIDEPRESSANT-LIKE EFFECT OF BUPROPION IN BEHAVIORAL PARADIGMS OF DESPAIR: POSSIBLE MECHANISM OF ACTION

Part-1: Involvement of nitric oxide (NO) signaling pathway in the antidepressant action of bupropion, a dopamine reuptake inhibitor

The present study was undertaken to elucidate the neurochemical basis of antidepressant action of bupropion [(±)-α-t-butylamino-3-chloropropiophenone], a dopamine reuptake inhibitor. The neurochemical changes were correlated with the behavioral changes. The involvement of L-arginine-nitric oxide (NO)-cyclic guanosine monophosphate (cGMP) signaling pathway in the antidepressant action of bupropion was investigated besides its actions on brain norepinephrine, dopamine and homovanillic acid. Bupropion (10, 15, 20 and 40 mg/kg, i.p.) dose dependently inhibited the immobility period in mice in both forced swim test and tail suspension test. ED$_{50}$ values of bupropion in reducing the immobility period was found to be 18.5 and 18 mg/kg i.p., in forced swim test and tail suspension test, respectively. Bupropion (10, 20 and 40 mg/kg., i.p.) reversed the reserpine-induced behavioral despair also. When different doses (10, 15, 20 and 40 mg/kg., i.p.) of bupropion were tested for locomotor activity, it (15, 20 and 40 mg/kg., i.p.) increased locomotor activity. At 20 and 40 mg/kg doses the drug showed hypothermia. The neurochemical analysis of brain samples revealed that bupropion dose dependently (10-40 mg/kg., i.p.) increased the brain contents of dopamine and homovanillic acid in the whole brain. The levels of norepinephrine were also increased at 20 mg/kg dose. The antidepressant-like effect of
bupropion (20 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 (25 mg/kg., i.p.) [a specific neuronal nitric oxide synthase (nNOS) inhibitor] produced potentiation of the action of subeffective dose of bupropion (10 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 bupropion (10 mg/kg., i.p.) in the forced swim test. Furthermore, the reduction in the immobility period elicited by bupropion (20 mg/kg., i.p.) was also inhibited by pretreatment with sildenafil (5 mg/kg., i.p.) [phosphodiesterase 5 inhibitor]. The study indicated that bupropion possessed antidepressant activities in different animal models of depression through its dopaminergic and/or by modulating the L-arginine-nitric oxide (NO)-cyclic guanosine monophosphate (cGMP) signaling pathway.

Part-2. Possible involvement of sigma-1 (σ₁) receptors in the anti-immobility action of bupropion, a dopamine reuptake inhibitor

Sigma receptors particularly, sigma-1 subtype is known to modulate the release of catecholamines in the brain and may participate in the mechanism of action of various antidepressants. The present study investigated the possible involvement of sigma receptors in modulating the anti-immobility-like effect of bupropion (a dopamine reuptake inhibitor) using the forced swim test (FST) in mice. Bupropion produced dose-dependent (10-40 mg/kg., i.p.) reduction in immobility period and the ED50 value was found out to be 18.5 (7.34-46.6) mg/kg, i.p. (+)-Pentazocine (2.5 mg/kg., i.p.), a high-affinity sigma-1 receptor agonist, produced synergistic response when it was co-administered with a subeffective dose of bupropion (10 mg/kg., i.p.). On the contrary, pretreatment with progesterone (10 mg/kg., s.c.), a sigma-1 receptor antagonist neurosteroid, rimcazole (5 mg/kg., i.p.), another sigma-1 receptor antagonist
antagonist, or BD 1047 (1 mg/kg., i.p.), a novel sigma-1 receptor antagonist, reversed the anti-immobility effects of bupropion (20 mg/kg., i.p.). The various modulators used in the study did not show any effect per se on locomotor activity except bupropion which at higher doses (15-40 mg/kg., i.p.) significantly increased the locomotor activity. The results for the first time demonstrated the involvement of sigma-1 receptors in the anti-immobility effects of bupropion.

CHAPTER 3: ANTIDEPRESSANT-LIKE EFFECT OF VENLAFAXINE IN BEHAVIORAL PARADIGMS OF DESPAIR: POSSIBLE MECHANISM OF ACTION

Part-1: Antidepressant activity of venlafaxine in mouse forced swim test: Involvement of L-arginine-nitric oxide-cyclic guanosine monophosphate pathway

The involvement of L-arginine-nitric oxide (NO)-cyclic guanosine monophosphate (cGMP) signaling pathway in the antidepressant action of venlafaxine (dual reuptake inhibitors of both serotonin and norepinephrine) was investigated in mice. The antidepressant activity was assessed in forced swim test (FST) behavioral paradigm. Total immobility period was calculated during the period of six minutes. 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 (PDE) 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.

Part-2: Involvement of sigma-1 receptor modulation in the antidepressant action of venlafaxine

The present study investigated the possible involvement of sigma receptors in modulating the antidepressant-like effect of venlafaxine (dual serotonin and norepinephrine reuptake inhibitor) in the mice employing the forced swim test (FST). Immobility period in the forced swim test was recorded for a total period of 6 minutes. Venlafaxine produced dose-dependent (4-16 mg/kg, i.p.) reduction in immobility period. Pretreatment of mice with (+)-pentazocine (2.5 mg/kg., i.p.), a high-affinity sigma-1 receptor agonist, produced synergism with subeffective dose of venlafaxine (2 mg/kg., i.p.). On the contrary, pretreatment with progesterone (10 mg/kg., s.c.), a sigma-1 receptor antagonist neurosteroid, rimcazole (5 mg/kg., i.p.), another sigma-1 receptor antagonist, or BD 1047 (1 mg/kg., i.p.), a novel sigma-1 receptor antagonist, reversed the anti-immobility effects of venlafaxine (8 mg/kg., i.p.). The various modulators used in the study did not produce any changes in locomotor activity per se except venlafaxine which showed a significant increase in the locomotor activity at higher dose (16 mg/kg., i.p.) in mice. The results for the first time demonstrated that the anti-immobility effects of venlafaxine possibly involved an interaction with sigma-1 receptors.
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Part-3: Positive modulation by yohimbine (α2 adrenoceptor antagonist) of the antidepressant activity of fluoxetine or venlafaxine

Various studies have suggested that α2 adrenoceptors strongly affect monoaminergic neurotransmission by enhancing not only noradrenergic but also serotonergic firing rates. With this background in mind, the present study was undertaken to monitor the effect of co-administration of yohimbine (α2 adrenoceptor antagonist) with fluoxetine (selective serotonin reuptake inhibitor) or venlafaxine (dual reuptake inhibitors of both serotonin and norepinephrine) in forced swim test (FST) in male laca mice. Immobility period was recorded in mouse Forced swim test during the period of six minutes. Different doses of the fluoxetine or venlafaxine were administered 30 minutes before exposing the animals to the test procedure. In combination study, yohimbine (2 mg/kg, i.p.) was administered 15 minutes before the administration of different doses of fluoxetine or venlafaxine. Fluoxetine (5- 40 mg/kg, i.p.) or venlafaxine (2-16 mg/kg, i.p.) dose dependently inhibited the immobility period in mice. Addition of yohimbine (2 mg/kg, i.p.) potentiated the antidepressant action of fluoxetine or venlafaxine in mouse FST as the animal showed decreased in immobility period compared to fluoxetine or venlafaxine *per se* group, respectively. The present study not only demonstrated the association of alpha2 receptors in the antidepressant effect of fluoxetine or venlafaxine but also support its adjuvant therapy with other antidepressant drugs in mental depression.

CHAPTER 4: INVOLVEMENT OF SIGMA (σ1) RECEPTORS IN MODULATING THE ANTIDEPRESSANT EFFECT OF NEUROSTEROIDS (DEHYDROEPIANDROSTERONE OR PREGNENOLONE) IN MOUSE TAIL-SUSPENSION TEST

The present study investigated the effects of neurosteroids dehydroepiandrosterone sulfate (DHEAS) or pregnenolone sulfate (PS) on
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the tail suspension test in mice, and also the possible involvement of sigma (σ) receptors. Immobility time in the tail-suspension test was measured for a total period of 6 minutes. DHEAS (10 and 40 mg/kg., s.c.) or pregnenolone sulfate (40 mg/kg., s.c.) significantly reduced the immobility period without accompanying changes in the locomotor activity in mice. The effect of DHEAS (10 and 40 mg/kg., s.c.) and PS (40 mg/kg., s.c.) was reversed by BD 1047 (1 mg/kg., s.c.), a novel σ₁ receptor antagonist, progesterone (10 mg/kg., s.c.), a σ receptor antagonistic neurosteroid or rimcazole (5 mg/kg., s.c.), another σ, receptor antagonistic property, respectively. The treatments and their combination did not alter the motor activity in mice. These data suggested a role for the central sigma receptors, particularly sigma-1 (σ₁) receptors in the antidepressant-like effects of neurosteroids.

CHAPTER 5: VENLAFAXINE REVERSES CHRONIC FATIGUE-INDUCED BEHAVIORAL, BIOCHEMICAL AND NEUROCHEMICAL ALTERATIONS IN MICE

A state of chronic fatigue was produced in mice when they were forced to swim (inside a rectangular jar of specific dimensions) everyday for a 6 min session for 15 days. Immobility period was recorded on alternate days. The effect of venlafaxine, a dual reuptake inhibitor of serotonin and norepinephrine was evaluated in this murine model of chronic fatigue. Venlafaxine was administered daily and on the days of testing, it was injected 30 min before forced swim session. On the 16th day i.e. 24 hrs after the last dose of venlafaxine, various behavioral, biochemical and neurochemical estimations in the brain were carried out. There was a significant increase in immobility period in vehicle treated mice on successive days, the maximum immobility score reaching on the 7th day and sustained till 15th day. Behavioural parameters revealed hyperlocomotion, anxiety response, muscle in-coordination, hyperalgesia
and memory deficit. Biochemical analysis showed a significant increase in lipid peroxidation, nitrite and myeloperoxidase levels and a decrease in the reduced glutathione (GSH) levels in brain homogenates. Further, there was a decrease in adrenal ascorbic acid following chronic forced swim. The neurotransmitter estimations in the brain samples revealed a decrease in norepinephrine, serotonin and dopamine levels on chronic exposure to forced swim for 15 days. Daily treatment with venlafaxine (8 and 16 mg/kg, i.p. for 15 days) produced a significant reduction in immobility period and reversed various behavioral, biochemical and neurotransmitter alterations induced by chronic fatigue. Venlafaxine could be of therapeutic potential in the management of chronic fatigue.

CHAPTER 6: INVOLVEMENT OF DOPAMINE (DA)/SEROTONIN (5-HT) / SIGMA (σ) RECEPTOR MODULATION IN MEDIATING THE ANTIDEPRESSANT ACTION OF ROPINIROLE HYDROCHLORIDE, A D2/D3 DOPAMINE RECEPTOR AGONIST

Multiple lines of investigation have examined the role of dopaminergic pathway in mental depression. Chronic treatment with antidepressant drugs has been reported to alter dopaminergic neurotransmission, most notably a sensitization of behavioural responses to agonists acting at D2/D3 dopamine receptors within the nucleus accumbens. Recent clinical evidences have shown that ropinirole, a D2/D3 dopamine receptor agonist, augments the action of various standard antidepressant drugs in treatment-resistant depression. The present study was undertaken to elucidate the possible mechanism of antidepressant action of ropinirole employing various behavioral paradigms of despair supported by the measurements of neurochemical changes in the tissue contents of dopamine (DA) and serotonin (5-HT) in the whole brain using High-Performance-Liquid Chromatography (HPLC) with electrochemical detectors (ECD). In the mouse forced swim test (FST) or tail-suspension...
test (TST), ropinirole (1-10 mg/kg., i.p.) produced an S-shape dose-response curve in the percentage decrease in immobility period. Compared with vehicle, ropinirole (10 mg/kg., i.p.) had a significant anti-immobility effect without affecting locomotor activity. The reduction in the immobility period elicited by ropinirole (10 mg/kg., i.p.) in the FST was reversed by dopaminergic and sigma receptor antagonist, haloperidol (0.5 mg/kg., i.p.), and specific D₂ dopamine receptor antagonist sulpiride (5 mg/kg., i.p.), but not by SCH 23390 (0.5 mg/kg i.p), a D₁ dopamine receptor antagonist. Rimcazole (5 mg/kg., i.p.) (a sigma receptor antagonist), progesterone (10 mg/kg., s.c.) (a sigma receptor antagonistic neurosteroid), BD 1047 (1 mg/kg., i.p.) (a novel sigma receptor antagonist with preferential affinity for sigma-1 sites) also reversed the anti-immobility effect of ropinirole (10 mg/kg., i.p.). The neurochemical studies of whole brain revealed that ropinirole at 10 mg/kg., i.p. did not affect the tissue levels of dopamine but significantly increased serotonin levels. The study indicated that ropinirole possessed anti-immobility activity in FST by altering dopaminergic, serotonergic or sigma receptor function.

CHAPTER 7: ON THE MECHANISM OF ANTIDEPRESSANT-LIKE ACTION OF BERBERINE CHLORIDE

Berberine, an alkaloid isolated from Berberis aristata Linn. has been used in the Indian system of medicine for a variety of ailments as a stomachic, bitter tonic, antiamoebic and also in the treatment of oriental sores. Evidences have demonstrated that berberine possessed 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. Following the acute administration of berberine (5 mg/kg., i.p.), there was an increase in the levels of norepinephrine (31 %), serotonin (47 %) and dopamine (31 %) of the whole brain. The chronic administration of berberine (5 mg/kg., i.p.) for 15 days brought about a significant increase in the levels of norepinephrine (29 %), serotonin (19 %) as well as dopamine (52 %). But at higher dose (10 mg/kg., i.p.) of berberine, there was no change in the norepinephrine (12 %) levels, however, a significant increase in the serotonin (53 %) and dopamine (31 %) levels was observed. The antidepressant-like effect of berberine (5 mg/kg., i.p.) 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.). 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.
CHAPTER 8: ON THE ANTIDEPRESSANT-LIKE ACTIVITIES OF NEW MOLECULAR ENTITIES IN VARIOUS BEHAVIORAL PARADIGMS OF DESPAIR

The present study investigated the antidepressant-like activity of various New Molecular Entities (NMEs) (synthesized in collaboration with the Department of Chemistry, Panjab University, Chandigarh) employing the two validated (chapter 1) animal models of despair. Various substituted derivatives of phenylethylamine moieties viz. N-methyl-1,2,3,4-tetrahydroisoquinoline, 7-methoxy-N-methyl-1,2,3,4-tetrahydroisoquinoline, 6-methoxy-N-methyl-1,2,3,4-tetrahydroisoquinoline, 6,7-dimethoxy-N-methyl-1,2,3,4-tetrahydroisoquinoline were synthesized and tested for antidepressant-like activities in mouse forced swim test. A few selected molecules were also screened in tail-suspension test and reserpine-induced behavioral despair, respectively. The other behavioral profiles such as their effect on pentobarbitone-induced hypnosis, body temperature, locomotor activity and assessment of catatonia were assessed. It was found that molecules with cyclohexanone or camphor group at N-methyl-1,2,3,4-tetrahydroisoquinoline basic moiety showed greater degree of antidepressant-like activity as compared with the standard drugs. Further, addition of single methoxyl or two methoxyl groups at the phenyl ring enhanced antidepressant-like activity of these compounds.

CONCLUSIONS

Based on the extensive investigations carried out in the present study, the following conclusions are reached:

i. Behavioral despair test (both FST and TST) is a sensitive animal model to detect the antidepressant profiles of various classes of antidepressant drugs.
ii. Besides the two well-established neurotransmitters, namely, norepinephrine and serotonin, dopamine also plays a critical role in mental depression. Agents possessing dopamine reuptake inhibiting properties have a place in the management of mental depression. There is a potential for triple (NE, 5-HT and DA) reuptake inhibitors as antidepressant drugs.

iii. The L-arginine-nitric oxide-cyclic guanosine monophosphate pathway and sigma receptor modulation play a significant role in the explanation of antidepressant action of drugs like venlafaxine, bupropion, neurosteroids and berberine chloride.

iv. Herbal drugs like berberine chloride produce antidepressant actions by modulating the brain neurochemistry. Berberine can be used in clinical situations of mental depression.

v. Mental depression is one of the major health problems affecting population all over the world. There is a continuous effort to discover newer, safer and quick acting antidepressant drugs. The substituted phenylethylamine moieties showed a promising profile as antidepressant drugs. These NMEs can be further explored for their clinical use.