

The role of beta-adrenergic receptors in the mechanism of action of imipramine in forced swim test.

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ABSTRACT:

Noradrenaline (NE) is believed to play an important role in the pathophysiology of depression, and in the mechanism of action of antidepressant compounds. Reduction in central postsynaptic beta-adrenoceptors density has been shown to mediate the therapeutic long-term effects of antidepressants. In the current study, acute, sub chronic and chronic administration of either beta-adrenergic agonist (isoprenaline) or antagonist (betaxolol) alone or in combination with an antidepressant drug (imipramine) was used to investigate the role of beta-adrenergic receptors in the antidepressant effect of imipramine. Forced swim test was used as an acute model of depression. Data of this model suggested a strong relationship between the antimobility effect of imipramine and central beta-adrenergic receptors. Changes in β 1-adrenergic receptor levels may mediate its activity in this specific animal model of depression. In conclusion, data of the present work suggest that the antidepressant effect of imipramine is most likely mediated at least in part by β 1-adrenoceptor downregulation in mice exposed to forced swim test.

INTRODUCTION

Depression is a serious disorder in today's society, with estimates of lifetime prevalence as high as 21-28% of the general population in some countries (1, 2). Mechanism of depression is not completely understood. Disturbances in neurotransmission are the neurobiological hallmark of depression. Changes have been found in monoamine systems, such as 5-Hydroxytryptamine (5-HT), noradrenaline (NE), and dopamine (DA), as well as other systems, such as corticotropin releasing factor (CRF) and somatostatin (3). Depression is characterized with altered brain monoamine levels and most antidepressant drugs act by elevating these monoamines in the synaptic cleft. This is because most of the current antidepressants act on neurotransmitter systems by affecting three distinct processes: neurotransmitter degradation; neurotransmitter reuptake; and neurotransmitter binding. Inhibiting a neurotransmitter degradation or re-up-taking increases its concentration in the synapse. This surge in the monoamine neurotransmitter concentrations increases its binding at postsynaptic receptors, this lead to down regulation of post synaptic receptors (4). The currently available antidepressants can basically be grouped into: monoamine oxidase inhibitors (MAOIs); tricyclic antidepressants (TCAs); selective serotonin reuptake inhibitors (SSRIs), serotonin and noradrenaline reuptake inhibitors (SNRIs); noradrenaline reuptake inhibitors (NRIs), receptor agonists and antagonists (RAs); and monoamine releasing agents (MRAs). It is not well known how antidepressants work. This, highlights the fact that the monoamine systems in the brain have overlapping but also slightly different profiles, and most of the data are from animal studies and more work need to be done in a clinical setting, in depressed patients. Some of these

studies in healthy people looked at the effects of drugs that selectively increase noradrenaline, dopamine, or serotonin (5) (3) (6). Noradrenaline, dopamine, or serotonin can be depleted in healthy people without inducing clinical depression. It was reported that those neurotransmitters can be depleted in patients who are depressed before treatment and they don't become worse, even if they're only mildly depressed, or even if they're severely depressed, they don't worsen (7, 8) Therefore, there are many suggested mechanisms that have been considered by researchers. Most of new hypothesis based on some neuro-adaptation changes in the brain that could happen after prolonged use of antidepressant drugs such as down-regulation of receptors. This based on the suggestion that, enhanced sensitivity of presynaptic α 2-adrenergic (9), and postsynaptic beta adrenergic receptors (10). For that reason, it has been suggested that depression results from a dysregulation of the noradrenergic neuronal system and that antidepressants act to return the systems to a state of equilibrium (11). Clinical evidence has not consistently supported the hypotheses of specific abnormalities in presynaptic regulation of noradrenergic system activity or postsynaptic beta-adrenergic receptor function. A blunted growth hormone response to the alpha2-adrenergic receptor agonist, clonidine, has been found in depression, panic disorder, and substance abuse, suggesting that abnormalities in postsynaptic noradrenergic receptor function are not specific to an affective illness (12). Centrally active beta-1 and beta-2 adrenergic agonists produce antidepressant-like effects in several behavioral tests, suggesting that these receptors may be involved in the mediation of the effects of antidepressant drugs (13). Up-regulation of β 1 adrenergic receptors in the brain of depressed patients supports this hypothesis. Up-regulation

is a term used for the hypothesis that depression may be associated with an overall increase in the number of postsynaptic receptors. Up-regulation has proved to be difficult to demonstrate in depressed patients, but there is evidence that antidepressant treatment tends to reduce receptor numbers, this effect of antidepressants is sometimes known as receptor down-regulation. The phenomenon is now quite well documented for SSRIs, TCAs and MAOs, as well as for the physical treatment; electroconvulsive therapy (ECT) (14). Our hypothesis suggests that imipramine induces its long-term effect on depression through down-regulation of β 1-adrenergic receptors. To study this hypothesis, it is challenging to reflect depression state in animal model, because depression is not a physical disease, it is a disease that affects mood and physical activity of the patient, and this cannot be reflected on animal. Because of that researcher developed many models, which depend mainly on studying animal response to several induced stresses. These stress deepened models can be classified to acute stress models and chronic stress models. The present project was designed to investigate the role of β 1-adrenergic receptors in the mechanism of action of imipramine, as antidepressant in acute stress models of depression. To study receptor changes mainly in biochemical studies includes western blot for receptor protein assessment and RT-PCR to detect the changes of receptor mRNA expression, unfortunately these techniques are not available in our lab, according to that we used simple test which can be anticipate the amount of available β 1-adrenergic receptors, isoprenaline induced drinking test was adapted from that described in (15) (16). Simply this test depended on drink indication of isoprenaline through activation of brain β 1-adrenergic receptors, according to that any changes in receptor number by antidepressant or β 1-adrenergic receptor agonist or antagonist could affect the drink induction by isoprenaline. As acute stress model we used the famous forced swimming test (FST), which has been proposed by Porsolt (1977) (17) and is used in rodents as an experimental model of depression. The mouse FST model was widely used in screening antidepressants due to its simplicity and because it has been reported to be reliable across laboratories. FST is sufficiently specific, since it discriminates antidepressants from neuroleptics and anxiolytics (18).

MATERIALS AND METHODS

Animals and drug treatment

Animals used throughout this study were male albino mice weighing (25 ± 5 kg). The animals were bred in the animal house at Tripoli University, Libya. They were kept at constant room temperature ($26 \pm 2^\circ\text{C}$), with 12 hours dark/light cycle. Animals were fed with standard mice diet, (ALCO, Sfax, Tunisia). The animals were allowed food and water ad libitum. Drugs were dissolved extemporaneously in distilled water, and administered intraperitoneally (i.p), in a constant volume of 5ml/Kg body weight. All drugs were freshly prepared.

Force Swim Test

In the present study, each mouse was individually forced to swim for 6 min in a vertical glass cylinder (height, 25 cm; diameter, 15 cm) containing 15 cm of water maintained at 25°C , so that it could not escape or touch the bottom. The duration of immobility is recorded during the last 4 min of the 6-min testing period. Mice were judged immobile when they float in an upright position and make only small movements to keep their head above water.

Isoprenaline Induced Drinking Test

Isoprenaline induced drinking test was adapted from that described in (15) (16). Mice were randomly allocated into several sub groups; animals were weighed and treated with isoprenaline (15mg/kg; i.p.) before the test. Each group was divided to three subgroups. Each subgroup had free access to a bottle of water with a constant level of water and predetermined weight three hours after isoprenaline injection. Weighing bottles containing the water before and after each test measured water consumption. Water consumption of each mouse was calculated according to body weight according to following formulated equation:

Water consumption by each mouse in the sub groups =

$$\frac{\text{weight of mouse} \times \text{total water consumption of the subgroup}}{\text{total weight of subgroup}}$$

Statistical Analysis

Descriptive statistical analysis was applied, on the parameters of samples within each experiment, to find out whether the observed samples were normally distributed, using the non-parametric test Kolmogorov-Smirnov for goodness of fit. If the parameters were normally distributed, one-way ANOVA was applied to compare treatments. According to the homogeneity of variance, data were transferred in rank if homogeneity of variance did not permit direct ANOVA analysis. For multiple comparison Post hoc tests, additional LSD tests were performed, when appropriate, to detect any significant differences between the treated groups and the control group, and between the combined drugs and drug itself. The difference was considered to be significant at $P \leq 0.05$. All analysis was conducted using the SPSS program.

RESULTS

Chronic administration of betaxolol enhances imipramine effects on forced Swim test

To choose a dose of imipramine that causes a significant decrease in immobility time, mice were randomly assigned to three groups of five mice each. Imipramine (Sigma Aldrich, Germany) 10 mg/kg and 15 mg/kg, were injected i.p. 24, 5 and 1 hour before conducting the test (19). The control group received normal saline. Imipramine-treated mice spent less time in the FST compared to control animals ($P \leq 0.05$). This reduction in immobility time was dose-time-dependent (Figure 1). On the other hand, there was no significant difference

between the two doses of imipramine, Therefore, the dose of 10 mg/kg was chosen for all subsequent experiments.

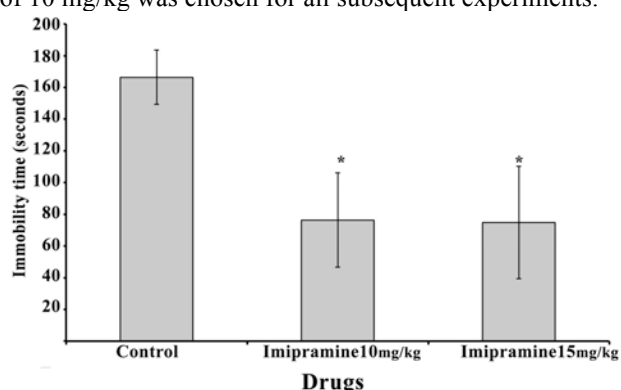


Figure 1. The immobility response to different sub chronic doses of imipramine in the forced swim test (n=5, each group). Responses to 15 mg/kg imipramine and 10 mg/kg were significantly different from the control group (*= $P \leq 0.05$).

From figure 1, it can be seen that sub chronic administration of imipramine has reduced the immobility time of tested mice. To investigate the anticipated roles of beta-adrenergic receptors in the mechanism of action of antidepressant, selective beta blocker (betaxolol, ALCON France) and non-selective beta agonist (isoprenaline, Sigma Aldrich, Germany) were used to test the anticipated role of beta adrenergic receptors in FST.. In this experiment, six groups (n=10) of mice were used: group 1, the control group, received normal saline 1 hour before test; group 2, injected with imipramine 24, 5 and 1 h before test; group 3, injected with isoprenaline (8mg/kg) alone (20); group 4, received betaxolol (2 mg/kg) alone (21); group 5 and 6, injected with imipramine in presence of betaxolol or isoprenaline, respectively. As it was expected, the immobility time of the control group” was highly significantly different from imipramine alone group ($P \leq 0.001$) (See Figure 2). Unexpectedly, acute administration of betaxolol or isoprenaline did not synergize nor antagonize the effect of imipramine in FST (Figure 2). Interestingly, the group treated with betaxolol alone (i.e group 4) showed significant reduction in immobility time compared to the control group ($P \leq 0.05$). In contrast, no significant differences were observed between mice treated with isoprenaline alone and the control animals ($P=0.527$). As described in the introductory paragraph, all the antidepressant drugs induce down regulation of beta-adrenergic receptors after chronic administration. In the present study, acute dose of betaxolol decreased the number of free receptors, this induces a temporary state seems to be receptor down regulation. This could explain immobility reduction induced by betaxolol. To clarify the anticipated role of beta receptors in the FST, receptor agonist and antagonist should be administrated chronically to induce receptor down regulation and up regulation respectively.

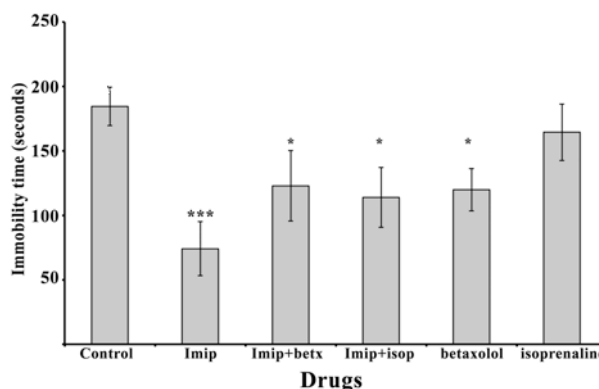


Figure 2. effects of acute doses of isoprenaline and betaxolol on the antimmobility effect of imipramine using FST in mice (n=10, each group). Responses to imipramine with betaxolol, imipramine with isoprenaline, and betaxolol alone, were significantly different from the control group ($P \leq 0.05$). Also, the response to imipramine alone, was highly significant different from that of the control group ($P \leq 0.001$). Imp=imipramine; betx=betaxolol; isop=isoprenaline.

In chronic drugs treatment study, mice were allocated into Six groups (n=9, each group): group1, imipramine alone (10mg/kg); group 2, imipramine plus betaxolol; group 3, imipramine plus isoprenaline; group 4, isoprenaline alone (8 mg/kg); group 5, betaxolol alone (2 mg/kg); group 6 was a control. Mice received drugs i.p once daily for twenty-one days (9-10 a.m.) and were forced to swim on day the twenty-second. As it was expected, the immobility time was highly significantly decreased by chronic administration of imipramine compared with the control group ($P \leq 0.001$, Figure 3). Interestingly, co-administration of betaxolol with imipramine showed the highest decrease in the immobility time compared to the other groups. This may confirm the anticipated role of beta adrenergic receptors in the effect of imipramine in the FST. To validate these findings another test (Isoprenaline-induced drinking test) was used to confirm receptor sub sensitivity after chronic drug treatment.

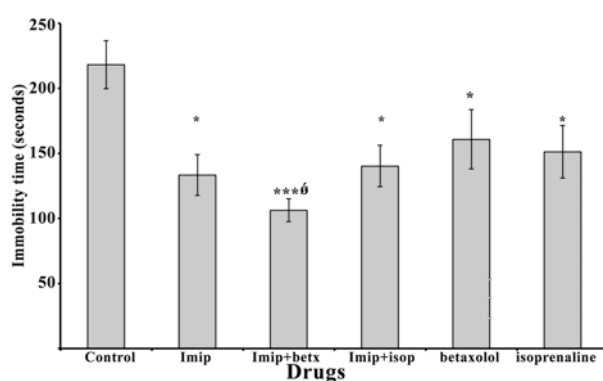


Figure 3. The effect of chronic administration of isoprenaline and betaxolol on the effect of imipramine in FST (n=9, each group). The response of the control group was highly significantly different from the imipramine group and imipramine with betaxolol group ($P \leq 0.001$), very significant different from imipramine and isoprenaline group and isoprenaline alone group ($P \leq$

0.01) and significant different from betaxolol alone group ($P \leq 0.05$). There was significant difference between imipramine group with betaxolol and betaxolol group ($\delta = P \leq 0.05$). imip=imipramine, isop=isoprenaline, betax=betaxolol.

Chronic administration of betaxolol antagonized the effect of imipramine on water consumption in isoprenaline-induced drinking test.

Isoprenaline-induced drinking test (described in section 2.3.) is an appropriate physiological indicator used to demonstrate beta-adrenoceptors sub-sensitivity following antidepressant treatment (20, 22). To test receptor sub-sensitivity, beta1-adrenergic antagonist should be administered in chronic dose to induce receptor upregulation. Mice were randomly divided into three groups (n=9, each group); Group 1; received saline and considered as the control group; Group 2; has received normal saline for fourteen days and then three doses of imipramine (24,5,1h) at day fifteenth; Group 3, received betaxolol 5 mg/kg twice daily (9-10 am,18-19 pm), for fourteen days, at day fifteenth, three doses of imipramine (24,5,1h) were administered. At day sixteenth, all groups were exposed to isoprenaline induced drinking test (figure 4).

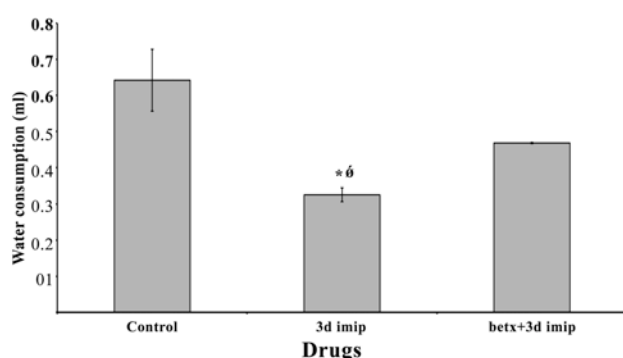


Figure 4. Responses of acute and sub-chronic administration of imipramine on the chronic administration of betaxolol on the isoprenaline induced drinking test. Responses to the control group was very significantly different from imipramine group ($P \leq 0.01$), and highly significant different from imipramine group ($P \leq 0.001$). Also, the decrease of water consumption induced by imipramine was very significant antagonized in Group 3 ($\delta = P \leq 0.01$). (n=9, each group). (imip=imipramine, betax=betaxolol, 1d=1dose, 3d=3doses).

As it was anticipated, isoprenaline was significantly induced drinking in the control group. This induction was highly significantly different from imipramine group (three doses 24,5,1h) ($P \leq 0.001$). Also, it was significantly higher than that of group administered betaxolol chronically (5mg/kg) followed by three doses of imipramine ($P \leq 0.01$). The decrease in water consumption induced by imipramine in the group injected with three doses of imipramine was very significantly antagonized in the animals pretreated with betaxolol two times daily for

fourteen days (5mg/kg) followed by three doses of imipramine ($P \leq 0.01$).

DISCUSSION

The neurochemical basis of depression now is regarded as being more complex and not the result of any one specific deficit. The areas of the brain implicated in depression are the forebrain and the limbic system (23). Since the 1960s, there has been a strong emphasis on role of noradrenaline in both the pathogenesis of affective disorders and in the mechanism of action of antidepressants (20). The centrally active beta-1 and beta-2 adrenergic agonists produce antidepressant-like effects in several behavioral tests, suggesting that these receptors may be involved in the mediation of the effects of antidepressant drugs (13). Likewise, long-term administration of several classes of antidepressants results in downregulation of the beta-adrenergic receptor, suggesting a common neuronal target for the effects of some antidepressants (26). The work described in this article focused on the role of beta1-adrenergic receptor in the antidepressant effect of imipramine. To approach the anticipated aim, the antidepressant effect of imipramine was investigated in acute animal model of depression.

In the present work, the (FST) was used to develop an acute stress model of depression in mice. FST is the most widely used pharmacological model for assessing antidepressant activity (18, 27). Until recently, research has focused on the ability of antidepressant drugs to decrease immobility in the FST paradigm (28). Exposure to the FST is also known to produce changes in the release of dopamine, noradrenaline, and serotonin in a variety of brain regions, and these effects interact with antidepressant drug treatments (29) (30). The FST is sensitive to the effects of a number of major classes of antidepressant treatments (18) (31). In FST, the relationship between serotonin and dopamine neurons may play a role in the action of antidepressant drugs, in addition, the interactions involving both GABAergic and serotonergic processes exist between benzodiazepine anxiolytics and some antidepressants (32). Noradrenaline and serotonin are both required for the process of the desensitization of central beta-adrenoceptor systems by antidepressants (15). It was reported (33) that the behavioral effects of imipramine in the FST are dependent upon noradrenaline uptake inhibition (33), which was mediated via presynaptic 5-HT_{1B} receptors (34) (35). Detke *et al.*, (1997) claimed that antidepressant drugs produce changes in the FST within 24h of the treatment (36). Also, Borsini and Meli, (1988) (18) emphasized on the administration of a drug twice or three times before discarding it as an antidepressant to be tested using the FST. However, the mechanism of action of antidepressants in the FST remains a paradox. Both pharmacological (from a wide variety of classes) and non-pharmacological treatments reduce immobility upon short-term administration, despite the long-term administration typically required to alleviate symptoms of depression in human populations. Although this is often a criticism of the test, it is conceivable that there is some

neurochemical adaptation to the stressful exposure that may be countered by antidepressants (37). In this work injecting mice with imipramine three times (24,5,1 hours before exposure to FST) was adopted throughout this study. Imipramine (10mg/kg) administered three times within 24h produced a reasonable antidepressant effect in FST (Figure 1). But, one acute dose of imipramine (one hour before test) produced insignificant effect on immobility time.

Further experiments were designed to investigate the role of beta-adrenergic receptors in the anti-immobility effect of imipramine in FST. Definitive evidence for a role of beta-receptors in the antidepressant effect of imipramine was sought using beta-adrenoceptor agonist and antagonist. On one part of this study, the anti-immobility effect of only one acute dose of the non-selective beta-adrenergic agonist "isoprenaline" alone, or in combination with sub-chronic dose of imipramine was examined. On another part of this study, the effect of a dose of selective beta1-antagonist "betaxolol" was investigated. Betaxolol was used in a dose which was considered to be sufficient to block beta- receptor subtype (13). Acute dose of isoprenaline significantly reversed imipramine – induced decrease in the duration of immobility. This result may in part agree with Parale (38) who, reported that the use of only one acute dose of isoprenaline prolonged the immobility duration in a dose related manner. However, a previous study (39), showed that (FST) in mice has failed to predict the antidepressant activity for drugs having also beta-adrenoceptors agonist activity. In contrast to isoprenaline' action, acute dose of betaxolol alone and in combination with imipramine produced a significant decrease in immobility time in FST. In agreement with this data, **Paul et al., (1990)** (33) claimed that, the behavioral effect of imipramine in FST is dependent upon noradrenaline uptake inhibition.

It is well established that very low level of neurotransmitters can lead to changes in the receptors themselves, even if there are no clinical signs. This often takes the form of increased receptor sensitivity or upregulation of receptors on the cell membrane, that may correlate with the start of depression. Antidepressant-induced alterations in beta-adrenergic receptor activity may, in some cases, be a function of receptor sensitivity at the time of drug administration (40).

In this work, no attempt was made to double the dose of isoprenaline as a trial to reverse the effect of imipramine. This is because, high doses of isoprenaline produces a well known cardiovascular side effects). In general, chronic administration of receptor agonist is known to down regulate this specific receptor type. By the injection of an acute dose of a receptor antagonist drug, it occupies the receptor and decrease the number of free receptors leading to a temporal state looks like downregulation of receptor beginning with the attachment of receptor and ended with the disappearance of the drug from the receptor. In this study, the observed decrease in the immobility time induced by only one acute dose of betaxolol alone may be due to beta1- adrenergic receptor blocking which produced a temporal state seems to be a

downregulation of the beta1-adrenergic receptor (figure 2). Nevertheless, in case of isoprenaline, it produced an effect discordant to that of betaxolol. It could be explained as the occupation of the beta-adrenergic receptors by isoprenaline may induce an effect similar to that produced by noradrenaline. However, even in combination with isoprenaline, imipramine showed a significant decrease in the immobility time (figure 3), which may be due to the effect of sub chronic dose of imipramine on beta-adrenergic receptor leading to a decrease in the effect of isoprenaline.

As expected, a decrease in immobility time was obviously seen in animals chronically treated with isoprenaline alone for 21 days. Also, chronic co-administration of isoprenaline with imipramine showed a very significant decrease in immobility time. Unexpectedly, chronic administration of betaxolol showed a significant decrease in immobility time when used alone, and a highly significant decrease in immobility time when combined with imipramine. This result may be explained at least in part by the dose range used in this study. Betaxolol was used in a dose, which just blocks the receptors (13). The significant difference in immobility time between the group treated with chronic dose of betaxolol alone, and the animals received a combination of chronic doses of betaxolol and imipramine might confirm the views regarding the unexpected results of betaxolol. It has to be noted that betaxolol is a beta1 selective adrenergic blocker with no partial agonistic effect and minimal membrane stabilizing activity. Its pharmacokinetic profile is characterized by a long serum half-life and excellent bioavailability (41). The unexpected results of betaxolol, which was mentioned above, may be reflected to the dose used of betaxolol, which may be considered insufficient to induce up regulation of central beta1 adrenergic receptor or may be due to pharmacokinetic property of betaxolol. Animals received the last dose of betaxolol 24 hours before the exposure to FST. This makes betaxolol available in the blood stream during the experiment time. Consequently, betaxolol blocks the receptors leading to the temporal state. However, the blockade of a steady-state agonist response to measure the potency of an antagonist might in some cases yield erroneous results and caution in the interpretation of the response should be taken (42). As proposed in this study chronic administration of betaxolol reversed the antimmobility effect of sub chronic dose of imipramine (figure 3).

Beta-adrenergic receptor down-regulation has been described as a common biochemical effect of chronic treatment with many but not all antidepressant drugs. Beta-receptor activation increases intracellular levels of cAMP followed by the activation of several protein kinases which in turn activate various transcription factors. Chronic treatment with many antidepressant drugs has been shown to alter constitutive depressor of eIF2 α phosphorylation (C-AMP responsive element binding protein (CREB) levels in several brain regions). While beta-receptor down-regulation by chronic antidepressant treatment has been a consistent finding, alterations of CREB levels have been observed in both

directions, similarly divergent findings have been reported for brain derived nerve growth factor (BDNF) a major gene targeted of CREB, where most findings suggest up-regulation at least at the message level following chronic antidepressant treatment (43). From all foregoing discussion, it appears that antidepressant mechanisms of action in the FST are different from each other.

This study concludes that, downregulation of beta1 receptors may mediate the effect of imipramine in the FST. Imipramine is rapidly and almost completely metabolized with the formation of desipramine (desmethylation), 2-hydroxy metabolites with subsequent glucuronide coupling (44) (45). The effect of imipramine in the FST may be reflected to its major metabolites desipramine; this could explain why desipramine and reboxetine had no effect in FST of dopamine- β -hydroxylase-deficient mice (46). The lack of effect of both drugs consistent with the hypothesis that both compounds elicit their effects through increasing synaptic noradrenaline via selective blocked of the noradrenaline transporter (47). As mentioned above NA reuptake inhibitors drugs may act through mediation of receptor downregulation.

One of the most common methods used for analysis of receptor activities are radio ligand binding and molecular cloning of proteins. Both techniques are not available in our laboratory. Therefore, the possible downregulation of beta- receptors was investigated using isoprenaline-induced drinking test. This test aimed to investigate the behavioral consequences of beta1- adrenoceptors sub-sensitivity by determining whether isoprenaline- induce drinking would be reduced by imipramine alone or in combination with betaxolol or isoprenaline. Our results has confirmed that the administration of an acute of isoprenaline (15mg/kg) induces drinking in mice. This increase was significantly different from animals received a chronic dose of isoprenaline (8mg/kg). Moreover, results presented in Figure 4 showed that the administration of betaxolol (5mg/kg) followed by three doses of imipramine antagonized the decrease in water consumption induced by imipramine. This data is consistent with results from the FST (Figure 3), (i.e betaxolol also has significantly decreased the anti-immobility effect of imipramine. This date may support the hypothesis reported by Matrisciano (48) and others (49) (50) about the relation between imipramine activity in FST and changes in beta1-receptor levels proposed to mediate its activity in this specific animal model of depression. Matrisciano (48) revealed that the administration of imipramine induced down regulation of beta-adrenergic receptor in the hippocampus after 15 and 21 days. Moreover, **Crissman**, (13) suggested that the discriminative stimulus effects of isoprenaline are mediated primarily via beta-1 adrenergic receptors. This provides a functional model for activation of central beta-1 adrenergic receptors, permitting further characterization of the role of this receptor subtype in the mechanism of action of antidepressant drugs. It was reported that isoprenaline produced a dose-dependent decrease in the

activity of an endogenous, specific inhibitor of cAMP-dependent protein kinase (type I inhibitor) in rat hippocampus, brain stem and pineal gland. Prolonged, 21-days treatment with some antidepressants (imipramine, nomifensin and mianserin), markedly reduced the response of the type I inhibitor activity to isoprenaline (51). Therefore, results of the present work indicated that one of the major needs is a better understanding of depression and estimation of circumstances that mediate abnormalities in mood. It would be interesting to conduct further studies to explore the role played by noradrenergic system in depression and antidepressant action by using other animal models of depression, including chronic stress models of depression.

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