Roles of β-adrenergic receptors on the mechanism of action of imipramine in chronic mild stress model of depression.

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ABSTRACT

The controversy theories of depression lead to several new hypotheses about antidepressant mechanism of action. Our date from the previous work indicated that beta receptors were involved in the mechanism of action of imipramine in the acute mode of depression the forced swim test). Nowadays, the well-established chronic model of depression, the chronic mild stress (CMS), invented by P Willner, is widely used in the screening of antidepressant activity. In this study, the involvement of β-adrenergic receptors in the mechanism of imipramine action was studied. Data from this research indicated that β-adrenergic receptors were not involved not only in the effect of imipramine effect on the reduction of cortisone level that induced by CMS model of depression, but also they had no effects on the effects of imipramine in the state. In conclusion, β-adrenergic receptors could be involved on imipramine activity of motivation enhancing not on the reduction of stress states.

Key words: Beta-adrenergic Receptors; imipramine; Depression; chronic mild stress; TCA.

Introduction

Mechanism of depression is still not completely understood; still the disturbances in neurotransmission are the neurobiological hallmark of depression (1), the mono-amine theory was created according to these mono-amine neurotransmitter disturbance and the mechanism of antidepressants drugs. In the last decades, a new concepts has been made against the mono-amine theory of depression, this include, many wonders about how norepinephrine and dopamine, or serotonin, or both can be depleted in healthy people without inducing clinical depression. Also 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 (2 - 4). On the other hand, the understanding of mechanisms of antidepressant action has evolved over time. The strong antidepressant activity of the tricyclic antidepressants (TCAs) has supported the role of both noradrenaline and serotonin (5-HT) in depression and the mechanism involved in antidepressant action (1). For many years, the main problem in the studying of depression and antidepressant drugs is how to reflect the depression state in the experimental animals. The animal behaviors of interest are not simply analogous (i.e., mimicking a human behavior) but homologous (i.e., resembling a human behavior, because

they serve a similar function and are activated by similar biological processes). Homologous models therefore permit testing of hypotheses. The wide spectrums of disruptions that characterize depression highlight the difficulty posing researchers to mimic the disorder in the laboratory. Stress plays a pivotal role in the pathogenesis of many psychiatric and medical illnesses (5). Individuals, however, display a heterogeneous susceptibility to the effects of stress. When an environmental stressor is used as the laboratory stimulus, the similarity of both the stressor and its outcomes are critical to any valid application of the model to an understanding of human behavior (6). In our previous data (7), it is confirmed that beta-adrenergic receptors is involved in the mechanism of action of imipramine in forced swimming test (acute stress model of depression). According to that, we conduct further studies to explore the role played by noradrenergic system in depression and antidepressant action by using another animal model of depression, chronic mild stress model of depression. The chronic mild stress (CMS) model shows high promise for the future (8), has a great many positive features, and is probably the most valid animal model of depression currently available (9). The procedure involves relatively continuous exposure of rats or mice to a variety of mild stressors, such as periods of food and water deprivation, small temperature reductions, changes of cage mates, and other similarly

innocuous manipulations. Over a period of a weeks of chronic exposure to the mild stress regime, mice, gradually reduce their consumption of a preferred" dilute sucrose solution, and in untreated animals this deficit persisted for several weeks following the cessation of stress (10). One of the core symptoms of human depression is anhedonia, represented by the loss of interest or pleasure in daily activities (11). Mice submitted to a regimen of chronic, mild, unpredictable stress exhibit behavioral deficits consistent with a loss of responsiveness to reward, such as decreased sucrose consumption (12), decreased ability to associate rewards with a distinctive environment, and decreased sensitivity to rewarding electrical brain stimulation. Normal behavior can be restored by chronic treatment with tricyclics (13). Moreau (14) reported that chronic stress-induced anhedonia in rodents, as an original animal model of human depression combining convergent elements of biological, etiological, symptomatological and therapeutic validity. Also animals exposed to CMS show an advanced phase shift of diurnal rhythms, diurnal variation, with symptoms worst at the start of the dark (active) phase, and show signs of increased activity in the Hypothalamic Pituitary Axis (HPA axis), including adrenal hypertrophy and corticosterone hyper-secretion (15). Imipramine reverses both the decreases of sucrose consumption and the increases of corticosterone level that induced by CMS. But the full mechanism of this effect is not well understood, from our earlier work we approved that the effect of imipramine in the forced swim test could be through it is effects on the down regulation of β-adrenergic receptors. In this study we will manipulate the role of β-adrenergic receptors in the mechanism of action of imipramine on more complicated chronic stress model of depression (The Chronic Mild Stress).

Materials and method

Animals

The animals used through out this study were male albino mice weighing (25+5g). The animals were breed in the animal house. They were kept at constant room temperature (26+2cº), with 12 hours dark/light cycle. Animals were fed standard mice diet, (obtained from ALCO, Sfax Tunisia). The animals were allowed food and water ad libitum. In all experiments animals were divided to 7 groups of 10 mice per group and subjected to different treatments. (Group1, Stress Control. Group2, No stress control. Group3, imipramine. Group4 imipramine+betaxolol. Group5, imipramine+isoprenaline. Group6,betaxolol only. group7, isoprenaline only)

Drugs and chemicals

Table.1. Chrnic mild stress protocol.

Imipramine HCL and Isoprenaline hemi sulfate were purchased from Sigma Aldrich, Germany. Betaxolol was obtained from ALCON France. ELISA kit was purchased from Neogen-corporation, USA. Hydrochloric acid and Ethyl ether were obtained from BDH limited Poole, England. Nitrogen gas was obtained from Al-Falah gases, Tripoli Libya. Deionized water to dilute wash buffer and extraction buffer was obtained from Al-Briga oil-company.

Equipment

The chronic mild stress (CMS) test was developed using a box made up of wood consisted of 120cmx240cm with height90cm, with heater the box closed and the heater connected to thermostat which keep temperature at 40cº, also 400 W flashing lamp, small 12cmx3cm tubes and reversed recorder with high power speaker were used. Microplate reader with 650 nm filter. Was obtained from biotechnology research center, Tripoli –Libya.

Drug administration

Drugs were dissolved extemporaneously in distilled water, and administered interapertoneally (i.p), in a constant volume of 5ml/Kg body weight. All drugs were freshly prepared.

Chronic mild stress model
Chronic mild stress protocol.

The Chronic mild stress (CMS) regimen was performed as previously described **(16, 17).** Mice were subjected to several mild stressors, which occurred in both the light and dark periods of the day during three consecutive weeks, as shown in table1. The weekly regimen consisted of one period of water deprivation for 7 hours, one period of continuous overnight illumination (200 W flashing lamp), two 17-hour periods in a soiled cage (1000 ml water) poured on sawdust bedding, two periods of 1 h confinement in a small 12 x 3 cm tube, three periods (2 and 3 h) of intermittent sound (reversed recorder with high power speaker, the unpredictable voice play for 10 sec and stop for 5 sec) and three periods (2 and 4 h) of exposure to heat at 40cº in wooden box (120x240cm with height 90cm, with heater the box closed and the heater connected to thermostat which keep temperature at 40º). Unstressed mice were kept in the same experimental room, but were not exposed to stressors. At day twenty-one, mice were subjected to the open field test and in day twenty- two animals were killed for further analysis.

Day no	Chronic mild Stress protocol "stressors"
0	Anhedonia Test
1	2h intermittent sound + overnight illumination
2	2h of exposure to heat 40Cº +17-hour periods in a soiled cage
3	I 2h intermittent sound+ confinement in a small 12 x 3 cm tube
4	2h of exposure to heat 40Cº+17-hour periods in a soiled cage
5	1h confinement in a small 12 x 3cm tube+ Anhedonia Test
6	3h intermittent sound+7h of water deprivation
7	2 h of exposure to heat 40C
8	3h intermittent sound + overnight illumination
9	4h of exposure to heat 40Cº +17-hour periods in a soiled cage
10	1h confinement in a small 12 x 3cm tube+ Anhedonia test
11	4h of exposure to heat 40Cº+17-hour periods in a soiled cage
12	2h intermittent sound+ confinement in a small 12 x 3 cm tube
13	3h intermittent sound
14	4 h of exposure to heat 40Cº+7h of water deprivation
15	1h confinement in a small 12 x 3 cm tube+ Anhedonia Test
16	2h intermittent sound + overnight illumination
17	4h of exposure to heat 40Cº +17-hour in a soiled cage
18	2h intermittent sound+ 1h confinement in a small 12 x 3 cm tube
19	2 h of exposure to heat 40Cº+1h confinement in a small 12 x 3 cm tube +7h of water deprivation
20	3h intermittent sound +17-hour in a soiled cage
21	Anhedonia Test

SUCROSE CONSUMPTION TEST

Sucrose consumption test **(16)** was carried out at days 0,5,10,15 and 21 (at 09:00 am.). After 3 h of food and water deprivation, the 2% sucrose solution was presented for 1 h. Fluid consumption was measured by weighing bottles containing the sucrose solution before and after each test session. Each group of animals is divided in to three sub groups (three mice each). As sucrose consumption depends on body weight, this variable was expressed according to body weight.

Sucrose consumption of each mouse in sub groups =

$$\frac{\text{Weight of mouse xTotal sucrose consumpof subgroup}}{\text{Total weight of subgroup}}$$

Open field test

Locomotor activity was studied using an open field test **(18)**. The open field apparatus is a square area (45x45cm) with a glass floor and outer square area (height 20cm) to prevent the mice from escape out from the apparatus. There are vertical and horizontal photocell detectors, in two side of the box, which received infrared light from the opposed side of the box. The photocell box is connected to a recorder, which, detect the number of movement of the animals during specific time. The open field test was performed in a closed room under a low level of illumination**,** under constant conditions such as temperature and humidity. Experiment time was fixed between 9am-1pm. Each animal was placed at the center of the apparatus and allowed for four minutes **(19)**. The ambulatory and non-ambulatory movements were recorded automatically. The animals were tested only once and did not have any prior exposure to the open field.

ELISA assay for serum corticosterone measurement
Contents of ELISA kit

The ELISA kit contain "EIA buffer": 30mL. Provided to dilute enzyme conjugate and corticosterone standards. Wash buffer 10X: 20 mL (dilute 10 fold with deionized water). Diluted wash buffer is used to wash all unbound enzyme conjugate, samples and standards from the plate after the one-hour incubation. Substrate: 20 mL. stabilized 3,3', 5,5'Tetramethylbenzidine (TMB) plus Hydrogen Peroxide (HP2) in a single bottle. It is used to develop the color in the well after they have been washed. Substrate was kept refrigerated; Extraction buffer 5X: 30 mL. (Dilute 5fold with deionized water). This buffer is used for diluting extracted and non-extracted samples. Corticosterone enzyme conjugate: 150 µL. Corticosterone horseradish peroxidase concentrate; Corticosterone standard: 100 µL. Corticosterone standard provided at concentration of 1µg/mL in methanol; Corticosterone antibody coated plate: A 96 well Costar TM Microplate with anti-Corticosterone rabbit antibody precoated on each well"

Extraction of Corticosterone

Animals exposed to CMS were slaughtered at day twenty-two. Trunk blood was collected in clean glass test tube and immediately reversed upside down for three times and left for 30 minutes for complete coagulation. After that blood was centrifuged for 15 minutes at 2400g. Serum collected and stored at -20 Cº for ELISA corticosterone assay. Extraction of Corticosterone by ELISA kit was performed according to the instructions of the company. Using a micropipette, 100µL of plasma was transferred in to a glass tube (10x75 mm) and 1mL of ethyl ether was added. The tube was exposed to vortex for 30 seconds and then the phases were allowed to separate. The organic phase was transferred into a clean glass tube and the solvent was evaporated with a stream of N2. The residue was dissolved in 100 μL of diluted extraction buffer (extraction buffer was diluted 5-fold with deionized water before use and any precipitant present brought into solution before dilution). The extract was diluted 100-fold by adding 10 μL of the above extract into 990 μL of diluted extraction buffer. 50 μL of finally obtained sample was assayed in duplicates. The values obtained were, multiplied by 100 to give final *ngl*mL concentrations.

Preparation of standards

The preparation of standards were performed according to the instructions of the company and used as seen in scheme I. Standard A, (1µg/ml; pure corticosterone) considered as a stock solution to prepare other standards (B, C and D) by serial dilution.

Standard B, prepared by adding 20 µl of standard A to 980 µl of EIA buffer and mix to give 20ng/ml. Standard C prepared by adding 200µI of standard B to 1.8 ml of EIA buffer and mixed to give 2ng/ml. Standard D prepared by adding 200 µI of standard C to 1.8 ml of EIA buffer and mixed to give 0.2ng/ml.

Table.2. Scheme I. The preparation of standards

Standard	ng/ml	EIA buffer (ml added)	B standard ml	C standard ml	D standard ml
S0	0	As is	-	-	-
S1	0.05	750	-	-	250
S2	0.1	500	-	-	500
S3	0.2	-	-	-	As is
S4	0.5	750	-	250	-
S5	1	500	-	500	-
S6	2	-	-	As is	-
S7	5	750	250	-	-

B, C and D, standards prepared form a stock solution (standard A) provided by the manufacturing company; S 0-7, standards prepared from standards B, C and D, and EIA buffer.

ELISA assay of samples and standards

A microplate with 96 well Coaster was used. Corticosterone enzyme conjugate was diluted by adding 1µI of enzyme conjugate into 50µI total volume of EIA buffer for each well assayed. For the whole plate, the solution was mixed thoroughly. 50 µI of standards (S) or unknown (U) were added to the appropriate wells in duplicate. 50 µI of the diluted enzyme conjugate was added to each well. The plate was shaken gently, and covered with plate cover and incubated at room temperature for one hour. Concentrated wash buffer was diluted with deionized water (i.e. 20 ml of wash buffer plus 180ml of deionized water) and mixed thoroughly. After incubation, the contents of the plate were dump out. The contents were tapped out thoroughly on a clean lint-free towel. Each well was washed with 300 µI of the diluted wash buffer for a total of three washings.150 µI of substrate was added to each well and the plate was shaken gently before the incubation at room temperature for 30 minutes. 100 µl of 1 N HCI was added to each well to stop enzyme reaction. The plate should be shaken gently before taking a reading to ensure uniform color throughout each well. Plate was read at 450 nm. A standard curve was plotted and the concentrations of the samples were estimated from the curve.

Calculation

The average of two S_o values is considered as B_o value (S1 becomes B1' etc.). The averages of each standard absorbance value (known as B1through B7) was divided by the B_o absorbance value and multiplied by 100 to achieve percentages. And the averages of each sample absorbance value were divided by the B_o value, and multiply by 100 to achieve percentages (see.table.3). To determined the concentration of corticosterone of the samples in diluted buffer, the standard curve was applied by plotting the %B/Bo for each standard concentration on the ordinate (y) axis against concentration on the abscissa (15) axis. The curve-fitting routine was used to draw the curve (i.e. linear regression) and the concentration of each sample was determined by regression between standard concentrations (%B/Bo) and the plotted equation (using Microsoft Excel – version software package program). Cross point between curve line and y-axis was chosen as "100" to exclude any mince results (see table.4). Readings of corticosterone concentration in diluted extract) multiplied by 100 to obtained corticosterone concentration by ng/ml of serum.

Statistic analysis

In the estimation of serum Corticosterone, Linear regression was applied for the standard solutions from which, the concentration of sample were calculated by regression curve equation, using Microsoft excel version software package. 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 Kolmogorove-smirnov maximum deviation test for goodness of fit. If the parameters were normally distributed, applying one-way ANOVA compared treatments. According to the homogeneity of variance, data were transferred in rank if homogeneity of variance did not permit direct ANOVA analysis. For multiple compression

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 $P \leq .05$. All analysis was conducted using the SPSS (software packing version eleven) for IBM compatible computer.

Results

Isoprenaline and betaxolol have not modify imipramine reduction on corticosterone level of mice exposed to chronic mild stress.

The average absorption at 450nm for serum samples collected from mice received different treatments and exposed to CMS presented in table 1. The $\%B/B_0$ was calculated as described above (table.4 and Figure.1).

Table 3. The average absorption at 450nm for serum samples collected from mice received different treatments and exposed to CMS and no stress control.

St conc. ng/ml	St absorb	Stress control	No stress control	betax	isop	Imip +beta	Imip +isop	imip
0	2.446	1.276	1.983	2.31	1.523	1.99	1.822	2.24
0.05	2.318	1.395	2.28	1.98	1.634	1.829	0.873	1.993
0.1	2.167	1.368	1.846	2.1	2.037	1.989	2.21	2.294
0.2	1.969	1.311	1.45	2.37	2.097	2.119	1.675	1.641
0.5	1.658	1.938	1.823	1.84	2.272	2.272	2.013	1.985
1	1.218	1.959	1.86	1.98	1.186	1.46	1.245	1.593
2	0.927	1.178	1.865	1.89	1.106	1.144	2.143	1.424
5	0.828	1.067		1.89		1.504	2.027	1.32
		1.346		1.56				

St, standard; imip, imipramine10mg/kg; isop, isoprenaline8mg/kg; ,betax, betaxolol5mg/kg

Table.4. The percentage of B/Bo calculated for serum samples collected from mice received different treatments and exposed to CMS and no stress control.

st conc. ng/ml	st absorb	Stress Control	No stress control	betax	isop	imp+ betax	imip+ isop	imp
0	100	52.1668	94.603	74.48	62.264	91.578	81.071	81.357
0.05	94.766	57.031	80.948	35.69	66.802	81.479	93.213	74.775
0.1	88.593	55.928	85.854	90.35	83.278	93.785	75.470	81.316
0.2	80.498	53.597	96.892	68.47	85.731	67.089	59.280	86.631
0.5	67.784	79.231	75.224	82.29	92.886	81.152	74.529	92.886
1	49.795	80.089	81.071	50.89	48.487	65.126	76.042	59.689
2	37.898	48.160	77.514	87.61	45.216	58.217	76.246	46.770
5	33.851	43.622	77.514	82.86		53.965		61.488
		55.028	63.941					

B, the average absorbance value of S doublicate; Bo, value of absorbance for standard "So";
s, standard; imip, imipramine 10mg/kg; isop, isoprenaline 8mg/kg; betax, betaxolol5mg/kg.

Figure. 2 showed induction on corticosterone level in stress control group, compare to no stress control group. As expected imipramine group showed significant reduction in corticosterone level compare to stress control group ($P \leq .01$). This was not altered with eithe co-administration of this drug with betaxolol nor isoprenaline. The chronic mild stress model significantly increased serum corticosterone level. Mice treated with isoprenaline or betaxolol alone showed a serum corticosterone level make them insignificantly different from the control group, which was taking normal, saline, and exposed to stress ($P > 0.05$) (Figure. 2).
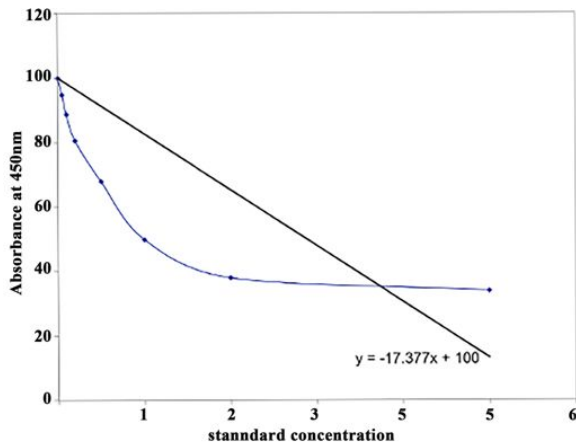
Figure.1. Regression between standard concentrations and %B/ B_0. B is the average of absorbance of S duplicate and B_0 is the average of absorbance of S_0 duplicate.

Equation of regression:

$x = (100-y)/17.377$

(y=sample %B/B_0, x=sample conc)

Sample concentration = (100- sample%B/B_0)/17.377

The concentration of each sample was determined by regression between %B/Bo and standard concentration. Using the equation of regression the sample corticosterone concentration was obtained and was multiplied by 100 (ng/ml) (table.5, figure, 2).

Table.5. Concentration of serum corticosterone in ng/ml of samples collected from mice exposed to CMS and no stress control.

Stress Control	No stress control	imip	imip+isop	imip+beta	isop	betax
275.2673	31.0558	107.2837	108.9306	48.46586	217.1553	146.8092
247.27	109.6364	145.1623	39.05502	106.5778	191.0402	370.0816
253.6223	81.40383	107.5189	141.1627	35.76122	96.22592	55.524
267.0328	17.88061	76.93368	234.3301	189.3933	82.10964	181.3941
119.5178	142.5743	40.93719	146.574	108.46	40.93719	101.8724
114.5771	108.9306	231.9774	137.8689	200.6863	296.4417	282.5607
298.3239	129.3992	306.3231	136.6926	240.4472	315.2634	71.28717
324.439	129.3992	221.6255		264.9154		98.57863
258.7983	207.5092					

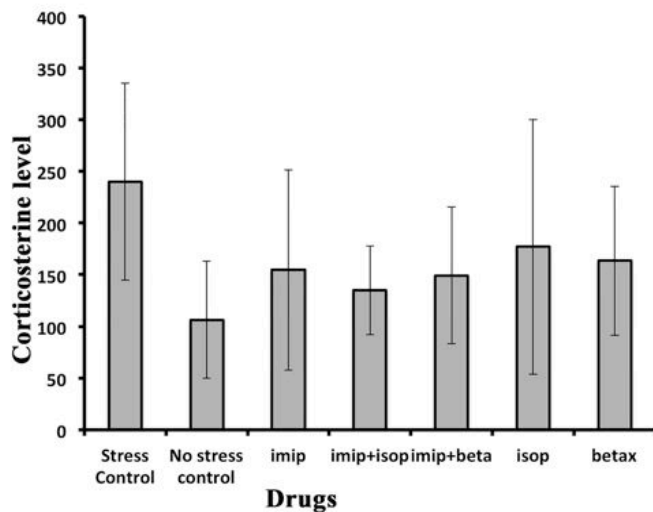*Imip, imipramine10mg/kg; ,isop, isoprenaline8mg/kg; betax,betaxolol5mg/kg*

Figure.2. Serum corticosterone level (ng/ml) in mice exposed to chronic mild stress and received different treatments (n=9, each group): (*= $P \leq .05$).

The effect of imipramine alone, and in combination with betaxolol or isoprenaline on the ambulatory and non ambulatory movements in open field test.

The ambulatory movement of mice was measure used open field test as described above. From open field test results in ambulatory movement, there was no significant difference between the all groups ($P \leq .05$) (Figure.3).

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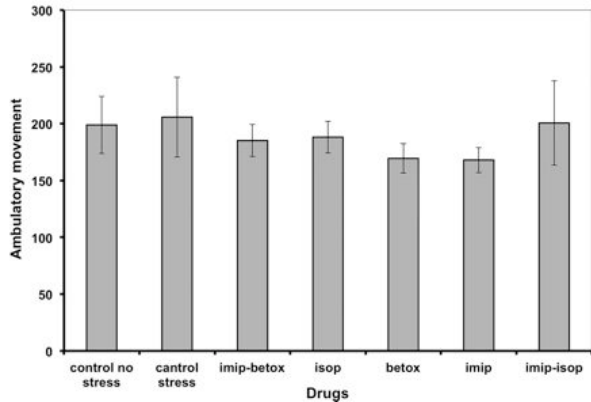
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Figure.3. The effect of chronic administration of imipramine alone, and in combination with betaxolol or isoprenaline on the ambulatory movement in open field test. (n=9, each group) The difference between groups was not significant. ($P \leq$.05).
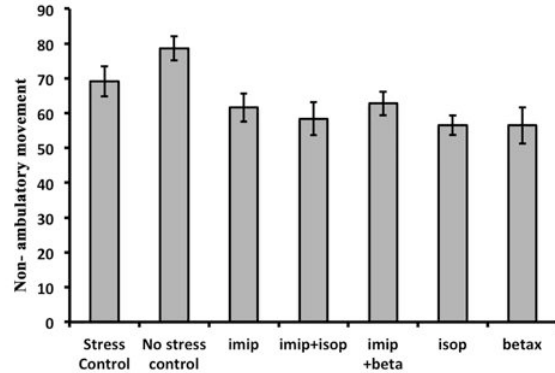

Figure.4. Effect of chronic use of betaxolol, isoprenaline and imipramine on the non- ambulatory movement in open field test (n=9, each group). Responses to "no stress C" group was very significantly different from imipramine, imipramine with betaxolol ($P \leq$.01), and highly significant different from all of the following groups, betaxolol and isoprenaline; imipramine and isoprenaline; ($P \leq$.001). All values are given as mean$\pm$SEM.

On the other hand, the non- ambulatory movement of the control group (received normal saline and didn't expose to stress) was very significantly different from imipramine alone group (61.62 ± 4.04), and imipramine with betaxolol group (62.75 ± 3.35; $P \leq$.01). The no stress control group was highly significantly deferent, from imipramine with isoprenaline group, isoprenaline alone group and betaxolol only. There was no significant difference between animals administered with normal saline and exposed to stress and the group which was taking normal saline without being exposure to stress (no stress C) (P=0.096). Also, figure.4 showed that the non ambulatory movement of isoprenaline alone group (56.57 ± 2.83) and betaxolol alone group were significantly different from that in stress control group ($P \leq$.05).

Modulation of anhedonia by betaxolol, isoprenaline and imipramine.
Control mice that exposed to CMS without any treatment, should insignificant difference in sucrose consumption

between day0 and day 21 (Figure.5.A). Animal treated with imipramine showed highly significant increase in sucrose consumption from day0 to day21 ($P \leq$0.0001) see (figure.5.B). The effect of imipramine was challenged by coadministration with isoprenaline. Figure.5.C shows a highly significant decrease in sucrose consumption measured at day0, when compared with that recorded at 5,10 and 15 day of the test ($P \leq$.001). Even though three is increased in sucrose consumption at day 21, but it still insignificantly compared to day0. On the other hand co-administration of betaxolol with imipramine (figure 5.D) did not completely compensate the effect of administration of imipramine on anhidonia, where it displayed increase in sucrose consumption from day0 up to day21 ($P \leq$0.01). While treating the animals with betaxolol alone did not affect the decrease in sucrose consumption that induced by chronic mild stress protocol (figure 5.F). In the same manner, admistration of isoprenaline alone did not induce the sucrose consumption. In soprenaline treatment mice, sucrose consumption reduced at day 5 and ended at day 21 (figure 5.E). (n=9, $P \leq$.001).
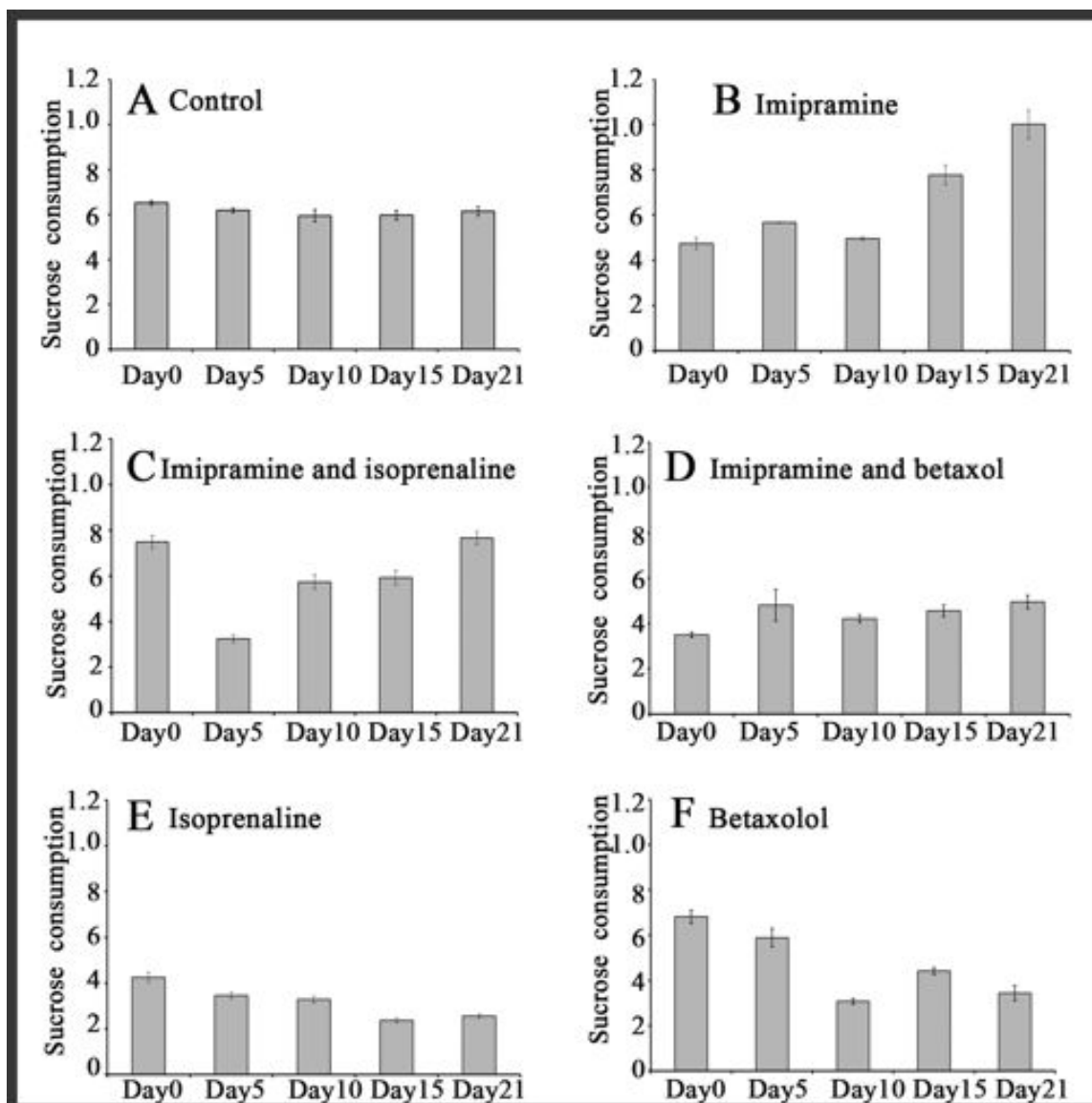
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Figure.5. Sucrose consumption at Day0, day5, day15, and day21 of mice subjected to chronic mild stress. A. stressed control mice during exposured to CMS (n=9, each group). **B**. Responses to imipramine in day 15 and in day 21 were highly significant different from that obtained at day 0. **C**. The effect of a combination of imipramine and isoprenaline on sucrose consumption during exposure of mice to CMS (n=9, each group). **D.** The effect of a combination of imipramine and betaxolol on sucrose consumption during the exposure of mice to CMS **E.** The effect of isoprenaline on sucrose consumption during exposure of mice to CMS. **F.** The effect of betaxolol on sucrose consumption during exposure of mice to CMS.. (All values are given as mean±SEM, n=9, each group, $P \le .001$).

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DISCUSSION

As discussed in our previous work **(7)**, for many decades noradrenaline plays critical roles in both the pathogenesis of affective disorders and in the mechanism of action of antidepressant medications**.** This could be due to the effect of long term administration of antidepressant drugs, which lead to downregulation of the beta-adrenergic receptor **(20)**. Crissman **(20)** suggested that, 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. Our data in acute stress model of depression indicated critical roles of beta- adrenergic receptors in the mechanism of anti immobility effects of imipramine in forced swim test **(7)**. Also in this manuscript, we focused on the role of beta1-adrenergic receptor in the antidepressant effect of imipramine in chronic stress model of depression, the chronic mild stress model of depression. As described above it increases the HPA axis activity and also induces anhedonic like state.

The studies conducted here were designed to examine the behavior of imipramine in relation to beta-receptor using the chronic mild stress (CMS) as a model of chronic stress model in mice. Chronic stress causes adaptive plasticity in the brain, in which local neurotransmitters as well as systemic hormones interact to produce structural as well as functional changes **(21).** Treatment with imipramine can reduce these behavioral changes but is only effective when given repeatedly prior to onset of CMS Reduction in preference for saccharin by repeated unpredictable stress **(22).** The body's response to different stressors is regulated largely by the HPA axis. Released Cortisol with other chemical messengers exerts widespread physiological effects throughout the body **(23)**. This study investigated the role of beta-adrenergic receptor in the relation between the upregulation of beta-adrenergic level and the elevation in the corticosterone level induced by stress. **Tafet (24)** reported that, the level of corticosterone was reduced to the levels of control group in rats subjected to 14 days of imipramine treatment. In agreement with**,** data presented in figure, 2 showed that imipramine alone decreased the levels of corticosterone significantly in mice exposed to chronic mild stress. Several brain pathways, including the hippocampus control the activity of the HPA axis **(25)**. Data presented in this study revealed that the chronic coadministration of isoprenaline or betaxolol with imipramine had no effect on imipramine-induced decrease in serum corticosterone level. Also, chronic administration of isoprenaline or betaxolol alone had no effect on chronic mild stress-induced increase in corticosterone level. It could be concluded that there may be no relation between the downregulation effect of imipramine on beta-adrenergic receptor and its effect on corticosterone level.

As described above locomoter activity usually measured by open field test. There is paradoxical conclusion of previous work on stress effect on the locomotor activity. It was reported that stress may increases locomotor activity **(26)** decreases motor activity (27)**,** or has no effect on locomotor activity **(28).** This paradoxical result is in agreement with the conclusion of **Harro (29)** who, claimed that stress had no clear effect on the open field locomotion in animals. **Strekalova and coworker (30)** found that the mean number of rearing was significantly decreased in the anhedonic group and the mean total distance moved in the open field did not differ between non-anhedonic, anhedonic, and control mice in low illumination**. Benelli (31)** showed that the chronic exposure to unpredictable mild stressors did not significantly modify ambulation and imipramine had no significant effect, either on ambulation or on rearing. Data presented in this study revealed that neither chronic mild stress nor any drug (imipramine, betaxolol, isoprenaline) used had any effect on ambulatory movement. Data of non ambulatory movement were not clear to explaine. The non ambulatory movement of mice exposed to chronic mild stress protocol were decreased but in nonsignificant rate when campared with non stressed control. In chronic mild stress test, the chronic administration of imipramine alone or in combination with isoprenaline or betaxolol decreased the non amobulatory movement when campared with control. In conclusion, chronic mild stress test had no effect on ambulatory and non ambulatory movement. Chronic administration of imipramine decreased the non amobulatory movement. But chronic administration of beta adrenergic agonist or antagonist had no effect on imipramine reduction on non ambulatory movement.

One core symptom of major depressive disorder is a decreased experience of pleasure or interest in previously enjoyed activities (anhedonia) such as work or hobbies, and is accompanied by decreased motivation or drive **(11).** It has been proposed that anhedonic deficits can also be induced in rodents by chronic stress **(12, 16, 22, 32).** Thus**, Katz and co-workers (32)** showed a decrease in sucrose intake in rats subjected to 21day strong stress that was interpreted as a sign of anhedonic deficit. To obtain a closer analogy to the human situation, **Willner** and his group used milder stressors such as soiled cage stress, presence of novel objects, restricted access to food, etc, and extended the stress exposure up to 3 months that produced a longer lasting decrease in sucrose consumption **(9, 16).**

A decrease in sucrose consumption in a limited access paradigm has been used previously to determine anhedonic deficits. Chronic mild stress is an antidepressant-responsive model for anhedonic symptoms of major depression **(33).** Catecholaminergic drugs would be the first choice for the anhedonia and decreased motivation **(34).** According to that, the effect on anhedonia by down regulation of beta-receptor with chronic administration of imipramine alone or in combination with chronic administration of isoprenaline or betaxolol was examined. Data of this work showed that, the administration of imipramine alone increased sucrose consumption at days 15, 21 when compared to day 0. This result is in agreement with the study of **Von Frijtag (35).** Imipramine alone reversed the decreased in sucrose consumption in mice subjected to chronic mild stress (Figure. 5B). Neither chronic coadministration of isoprenaline nor betaxolol with imipramine modulated the effect of imipramine (Figure. 5C, 5D). There was a significant decrease in sucrose consumption of isoprenaline alone group from day 0 to day 21 (Figure. 5E). Chronic coadministration of betaxolol with imipramine didn't alter the effect of imipramine on anhedonia (Figure. 5F). The sucrose consumption in imipramine with betaxolol group was significantly increased from day 0 to day 21. But chronic administration of betaxolol alone had no effect on chronic mild stress produced decrease in sucrose consumption. In case of stress control group there is no significant decrease in sucrose consumption from day 0 to day 21. It could be concluded that beta1-adrenergic receptors do not appear to be a part of the decreased in sucrose solution consumption in CMS model and may not participate in the effect of imipramine on anhedonia.

The hypothesis is that altered brain reward system function may be an underlying brain mechanism of the loss of pleasure/interest experienced in major depressive disorder **(36).** Researchers theorize that anhedonia may result from the breakdown in the brain's reward system, involving dopamine pathways **(37, 38).** Presence of a hypersensitive response is present in the brain reward system of depressed patients, which may reflect a hypofunctional state and may provide a novel pathophysiologic and therapeutic target for future studies **(38). O'Neill and Conway (39)** suggested that 5-HT1B might mediate the effects of imipramine**.** The activation of 5-HT1B receptors attenuates the effects of a dopamine-dependent behavior, and that activation of these receptors can oppose the behavioral effects of elevated mesolimbic dopamine transmission **(40).** Other studies **(41-43)** revealed that anhedonia associated with chronic mild stress involves a perturbation of activity at mesolimbic D_2 receptors. So it could be concluded that the effect of imipramine in anhedonia is due to its effect on central serotonergic system **(44).** Chronic mild stress causes a decrease in the sensitivity of postsynaptic D2 receptors, secondary to a chronic increase in dopamine release **(45).**

The imipramine effect in the anhedonic state may be due to its activation of 5HT1B receptor within the nucleus accumbens. This attenuates the effects of a dopamine-dependent behaviour, at mesolimbic D_2. In conclusion, our work in this manuscript and previously publish work indicated that, not only the noradrenergic system is involved in the mechanism of imipramine as antidepressant drug, but also it could involve the other monoamine neurotransmitters including dopamine and 5HT. From our data the increase in motivation induced by imipramine could be conducted to Downregulation of beta-adrenergic receptors. On the other hand it is effects in the reduction of HPA activity and in the anhedonic state may be due to it is action in dopaminergic and 5HT systems in the brain.

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