INVOLVEMENT OF SOME 5-HT RECEPTORS IN METHAMPHETAMINE-INDUCED LOCOMOTOR ACTIVITY IN MICE

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Abstract

Background

Effects of some selective 5-HT antagonists on methamphetamine-induced locomotor activity were investigated in male mice in order to study whether this effect of methamphetamine is selectively or at least partially, induced through stimulation of a specific serotonin receptor subtype.

Results

Methamphetamine (1.5mg/kg, IP) produced a significant increase in locomotor activity. Methamphetamine-induced hyperactivity was signficantly antagonized by NAN-190 (5-HT1A antagonist) at a dose of 4 mg/kg, IP, methiothepin (5-HT1B/1D antagonist) at a dose of 0.1mg/kg, IP. and mianserin (5-HT2C antagonist) at a dose of 8mg/kg, IP. On the other hand, methysergide (5-HT 2A/2B antagonist) at a dose of 1mg/kg, IP. and ondansetron (5-HT3 antagonist) at a dose of 0.5mg/kg, IP. potentiated the methamphetamine-induced hyperactivity. None of the above mentioned 5-HT antagonists altered the spontaneous activity of mice when it was administered alone.

Conclusion

The results of the present study indicate a possible role for tryptaminergic mechanisms, in addition to the catecholaminergic systems, in the locomotor stimulant activity of methamphetamine in mice. This role is possibly mediated through direct stimulation of some 5-HT receptor subtypes. Stimulation by methamphetamine of 5-HT 1A, 5-HT 1B/1D and/or 5-HT2C receptor subtypes may result in hyperactivity, whereas stimulation by methamphetamine of 5-HT 2A/2B and/or 5-HT 3 receptor subtypes may result in decreased activity. However, the latter effect is probably not observed because other stimulating systems are predominant.

KEY WORDS : methamphetamine, NAN-190, methiothepin, methysergide, mianserin, ondansetron, locomotor activity, 5-HT.

Background

Some aspects of locomotor activity and the stereotyped behavior induced by methamphetamine are probably a consequence of the central release of dopamine [1, 2], norepinephrine [3] and/or 5-hydroxytryptamine (5-HT) [4-6]. Other suggested mechanisms for methamphetamine's actions include reuptake inhibition of biogenic amines and inhibition of monoamine oxidase [7]. Both mechanisms may result in increased concentrations of the biogenic amines centrally and peripherally. In addition, methamphetamine may exert direct effects on central receptors for the biogenic amines.

The brain tryptaminergic systems are suggested to be associated with the symptomatology of major behavioral disorders. This may explain the effectiveness of 5-HT antagonists in some behavioral conditions. 5-HT produces an inhibitory action

on locomotor activity, but this inhibitory action is not only mediated through modulation of the tryptaminergic system, but is also mediated through modulation of dopaminergic as well as glutamatergic systems [8-12]. Methamphetamine increases central 5-HT levels more markedly than other psychomotor stimulants such as amphetamine or cocaine [13]. However it does not depress locomotor activity, and agents like p- chlorophenylalanine, which reduce 5-HT level, have been reported to increase activity [14, 15]. Therefore, it is likely that there are other mechanisms involving 5-HT in the locomotor stimulant effect of methamphetamine. With the advent of the new 5-HT receptor classification, methamphetamine's interaction with these receptors needs to be explored.

Molecular biological approaches have led to the identification of 14 distinct mammalian 5-HT receptor subtypes [16]. At present, the known 5-HT receptor subtypes have been grouped into multiple classes: the 5-HT_1 and 5-HT_2 classes of receptor are both G-protein coupled receptors with a seven- transmembrane-spanning-domain motif and include multiple isoforms within each class, while the 5-HT_3 receptor is a ligand gated ion channel with structural similarity to the α-subunit of the nicotinic acetylcholine receptor. The 5-HT_4, 5-HT_5, 5-HT_6 and 5-HT_7 classes of receptor, which all possess seven putative transmembrane-spanning domains, have been identified by molecular cloning and characterized biochemically, but have not yet been extensively studied electrophysiologically or operationally [17-19].

Therefore, the present study was designed to study whether the effects of methamphetamine are directly or indirectly induced through stimulation of specific 5-HT receptor subtypes. For this purpose, the effects of some selective 5-HT receptor antagonists have been studied on methamphetamine-induced locomotor activity.

Results

All figures presented show the time course-effect curves of drugs on the locomotor activity at 10 min time intervals. Activity recording started immediately after the second treatment. From preliminary experiments, methamphetamine at a dose of 1 mg/kg did not modify the spontaneous locomotor activity of male mice throughout the 2 hour observation period. Doses of 1.5 and 2 mg/kg of methamphetamine, however, significantly increased the spontaneous locomotor activity of mice; the latter dose was the more effective in enhancing activity of mice. The intermediate dose of methamphetamine (1.5 mg/kg) was selected for challenge with 5-HT antagonists in subsequent experiments.

The effect of NAN- 190 on methamphetamine-induced locomotor activity:

The effect of various doses of NAN-190 on methamphetamine-induced locomotor activity in male mice was explored. A dose of 4 mg/kg of NAN-190 was the least possible dose that significantly reduced methamphetamine-induced locomotor activity throughout the 2 hour observation period ($P<0.0\ 1$), as seen in Fig 1.

It is also clear from Fig 1. that NAN-190 at a dose of 4 mg/kg, when used alone (NAN 4 + saline groups), did not modify the spontaneous locomotor activity of male mice (saline + saline groups). NAN-190 at a dose of 4 mg/kg did not reduce the locomotor stimulant effect of a higher dose of methamphetamine (3 mg/kg), as seen in

Fig 2, indicating competitive antagonism by NAN-190 on methamphetamine-induced locomotor activity.

The effect of methiothepin on methamphetamine-induced locomotor activity:

From a series of doses of methiothepin, a dose of 0.1 mg/kg was found to be the least possible dose that reduced methamphetamine-induced locomotor activity. Methiothepin (0.1 mg/kg) significantly reduced methamphetamine-induced locomotor activity at time intervals (10-40) and (70-90)-min ($P<0.05$), as shown in Fig 3. That higher dose of methiothepin (0.1 mg/kg) did not modify the spontaneous locomotor activity of male mice when used alone as seen in Fig 3.

Methiothepin (0.1 mg/kg) failed to reverse the locomotor stimulant effect of higher doses (3 and 6 mg/kg) of methamphetamine (no Fig is presented). Moreover, methamphetamine (6 mg/kg) was found to be toxic (40 % of the tested animals died).

The effect of methysergide on methamphetamine-induced locomotor activity:

A dose of 0.5 mg/kg of methysergide did not change methamphetamine-induced locomotor activity, whereas a higher dose of methysergide (1 mg/kg) significantly increased it at time intervals (10-80) min, ($P<0.0$ 1), as shown in Fig 4. Doses higher than 1 mg/kg of methysergide produced similar effects to that of methysergide (1 mg/kg). Methysergide (1 mg/kg) did not modify the spontaneous locomotor activity of male mice when used alone (Fig 4).

Methysergide (1 mg/kg) did not alter the locomotor stimulant effect of a higher dose of methamphetamine (3 mg/kg), as seen in Fig 5, indicating competitive antagonism by methysergide on methamphetamine-induced locomotor activity.

The effect of mianserin on methamphetamine-induced locomotor activity:

The effect of various doses of mianserin on methamphetamine-induced locomotor activity in male mice was studied. Mianserin (4 mg/kg) did not change methamphetamine-induced locomotor activity, whereas a higher dose of mianserin (8 mg/kg) significantly reduced it throughout the 2 hour observation period ($P<0.0$ 1), as shown in Fig 6. The effect of doses higher than 8 mg/kg of mianserin on methamphetamine-induced locomotor activity were similar to that of the 8mg/kg dose of mianserin (no Fig is presented).

That dose of mianserin (8 mg/kg) did not modify the spontaneous locomotor activity of male mice when used alone (Fig 6). Competitive antagonism by mianserin of methamphetamine-induced locomotor activity was observed (Fig 7), since the locmotor stimulant effect of a higher dose of methamphetamine (3 mg/kg) was not reversed by mianserin (8mg/kg).

The effect of ondansetron on methamphetamine-induced locomotor activity:

Doses of (0.05 and 0.1 mg/kg) of ondansetron did not change methamphetamine-induced locomotor activity, whereas a higher dose of ondansetron (0.5 mg/kg), as seen in Fig 8, significantly increased it at time intervals (40-60) and (90-120)-min ($P<0.01$). Doses higher than 0.5 mg/kg ondansetron produced similar effects to that of

the 0.5 mg/kg dose of ondansetron (no Fig is presented). Ondansetron (0.5 mg/kg) did not modify the spontaneous locomotor activity of male mice when used alone (Fig 8). Competitive antagonism is also exhibited by ondansetron on the locomotor stimulant effect of methamphetamine (Fig 9).

Discussion

In this study, known selective receptor antagonists for 5 -HT (subtypes 1-3) have been used prior to methamphetamine treatment, and locomotor activity was subsequently evaluated. Prior treatment with some 5-HT antagonists (NAN-190, methiothepin and mianserin) caused inhibition whereas other antagonists (methysergide and ondansetron) caused potentiation of methamphetamine-induced locomotor activity in male mice. However, none of the doses employed of those selective antagonists for 5-HT receptors modified the spontaneous locomotor activity.

NAN-190 (5-HT_{1A} receptor antagonist) produced an inhibitory action on methamphetamine-induced hyperactivity. Similar findings have previously been reported [20]. This suggests that the 5-HT_{1A} receptor subtype is involved in the mediation of methamphetamine-induced hyperactivity. However, the mechanism underlying this involvement is not clear, but may be related to modulation of the presynaptic inhibitory autoreceptors, which are believed to reduce 5-HT release when they are stimulated. 5-HT_{1A} receptors reveal a dual localization: that is, they are situated both postsynaptically to serotoninergic neurons, for example in the spinal cord, hypothalamus, hippocampus and cortex as well as presynaptically as inhibitory autoreceptors on dendrites of raphe-localized serotoninergic perikarya [21]. These results may suggest a direct agonistic effect of methamphetamine on presynaptic 5-HT_{1A} receptors. The presynaptic 5-HT_1 autoreceptors are more sensitive in nature to both agonists and antagonists when compared with the postsynaptic receptors [22, 23]. The inhibitory effect of NAN-190 towards methamphetamine-induced hyperactivity is mediated pharmacologically rather than being a result of non specific sedation, since NAN-190 alone did not decrease the mobility of control mice [24]. Millan and Colpaert [20] did not exclude the role of dopaminergic and adrenergic pathways in the locomotor-activating actions of methamphetamine since prior treatment with a dopaminergic receptor antagonist (SCH23390), or an adrenergic receptor antagonist (alprenolol) also attenuated the methamphetamine-induced hyperactivity.

Like NAN-190, methiothepin (5-HT_{1B} /$_{1D}$ receptors antagonist) blocked methamphetamine-induced hyperactivity, probably through presynaptic 5-HT_1 autoreceptors. These findings also suggest the involvement of 5-$HT_{1B/1D}$ receptors in methamphetamine-induced hyperactivity. However, the evidence for the involvement of these receptors is not conclusive, since methiothepin is also known to block central and peripheral α-adrenoceptors as well as its ability to block the 5-HT_7 receptor [25, 26].

Involvement of 5-HT_{2C} receptor in the induction of methamphetamine-induced hyperactivity is also likely, since pretreatment with the 5-HT_{2C} antagonist mianserin resulted in blockade of the hyperactivity induced by methamphetamine. Mianserin is also known to block dopamine D_2-receptors [27, 28, 29], thus a role for dopaminergic systems in the hyperactivity induced by methamphetamine is also indicated. Similar findings have previously been reported [30].

In contrast to the antagonism of methamphetamine-induced hyperactivity by NAN-190, methiothepin or mianserin, the 5-$HT_{2A/2B}$ receptor antagonist methysergide and the 5-HT_3 antagonist ondansetron, both have the ability to potentiate methamphetamine-induced hyperactivity. Such an antagonism suggests an inhibitory role for 5-$HT_{2A/2B}$ and 5-HT_3 receptors on methamphetamine-induced hyperactivity, either through a direct stimulatory effect of methamphetamine on these receptors or through the released 5-HT by methamphetamine. It has been suggested that methysergide may mediate its effect through increasing the central dopamine function and activating the dopaminergic mechanisms in the mouse brain, since pretreatment with haloperidol (non-selective dopaminergic receptors antagonist) blocked methysergide-induced potentiation of methamphetamine-induced hyperactivity [31, 32, 33]. Alternatively, it may be related to the ability of methysergide to stimulate the ascending serotoninergic projections resulting in inhibition of serotonin release [34]. Ondansetron, on the other hand, may mediate its effect through modulating the firing of mesolimbic dopaminergic cell bodies [35].

Conclusions

The results of the present study indicate a possible role for tryptaminergic mechanisms, in addition to the catecholaminergic systems, in the locomotor stimulant activity of methamphetamine in mice. This role is possibly mediated through direct stimulation of some 5-HT receptor subtypes, since some of the known 5-HT antagonists (NAN-190, methiothepin and ondansetron) reversed, whereas others potentiated (methysegide and mianserin) methamphetamine-induced hyperactivity. Even competitive antagonism between these anagonists (except for methiothepin) and methamphetamine was demonstrated. Thus, stimulation by methamphetamine of 5-HT_{1A}, 5-$HT_{1B/1D}$ and/or 5-HT_{2C} receptor subtypes (which are blocked by NAN-190, methiothepin and mianserin, respectively), may result in hyperactivity. On the other hand, stimulation by methamphetamine of 5-$HT_{2A/2B}$ and/or 5-HT_3 receptor subtypes (which are blocked by methysegide and ondansetron, respectively), may result in decreased activity. However, the latter effect is probably not observed because other stimulating systems are predominant.

Methods

Animals:

Male Swiss albino mice (obtained from the Animal Care Center, College of Pharmacy, King Saud University) weighing 25-30 grams were used. The animals were housed, 10 mice per cage (35 x 25 x 15 cm) with woodchip bedding, under conditions of constant room temperature (23 ± 1^0C), humidity and light cycle (7 a.m. to 7 p.m.). They were given access to food (standard lab chow, Grain silos and flour mills organization, Riyadh) and water ad libitum. The experiments described in this study were approved by a local Ethical Committee for the Conduction of Animal Experiments.

Drugs:

Drugs used in this study were: methamphetamine hydrochloride (E.Merck, Germany), NAN-190 hydrobromide (Sigma, USA), methiothepin (Winlab, UK), methysergide (Research Biochemical International, USA), mianserin hydrochloride (Sigma, USA) and ondansetron hydrochloride dihydrate (Glaxo Laboratories, UK). All drugs were

dissolved in 0.9% NaCl solution. Doses are expressed as mg/kg of the salt. The dose volume administered was 10 ml/kg, intraperitoneally.

Experimental design:

In each of the experimental procedures planned, four groups were designated for each 5-HT antagonist study, as follows:

Group 1 (control group) first treatment was saline; second treatment was saline.
Group 2 first treatment was antagonist; second treatment was saline.
Group 3 first treatment was saline; second treatment was methamphetamine.
Group 4 first treatment was antagonist; second treatment was methamphetamine.

The first treatment was given 30 min before the second treatment. Appropriate drug dosages were determined from pilot experiments.

Procedure:

Locomotor activity was recorded by an activity meter (Optovarimex, Columbus, Ohio, USA). The activity cage of this instrument is equipped with horizontal and vertical sets of infrared photocells, which send continuous unseen light beams. The number of light beam interruptions due to the animal's movement inside the cage was automatically recorded. Each group of five mice was placed in the activity cage and counts of motor activity were recorded automatically every 10 minutes for two hours following saline or drug administration. Experiments were run between 10.00 a.m. and 3.00 p.m. under standard conditions of temperature, lighting and noise as was practicable.

Statistical analysis:

Statistical analysis of the results was performed by using one way ANOVA. For significant results, a post-hoc comparison between the means was done by Tukey-krammer test. The level of significance adopted was at $P < 0.05$.

Authors' contributions

Author 1 (OG) designed the experiments and wrote the manuscript. Author 2 (AA) revised the manuscript and and procured the grant. Author 3 (AA) performed the experiments. Author 4 (TE) advised and revised the statistical evaluation. Author 5 (OA) provided the facilities

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Figures

Figure 1 - Effect of NAN-190 alone (4 mg/kg, IP) and in combination with methamphetamine (1.5 mg/kg, IP) on the locomotor activity of male mice.

In all figures:
Each point represents the mean activity counts of 4 groups of mice.
n per group = 5 mice.
Vertical bars represent S.E.M
**: P<0.01, as compared to saline control.
***: P<0.001, as compared to saline control.
#: P<0.05, as compared to saline + methamphetamine group.
##: P<0.01, as compared to saline + methamphetamine group.
###: P<0.001, as compared to saline + methamphetamine group.

Figure 2 - Effect of methamphetamine (3 mg/kg, IP) alone and NAN-190 (4 mg/kg, IP) in combination with methamphetamine (3 mg/kg, IP) on the locomotor activity of male mice.

Figure 3 - Effect of methiothepin alone (0.1 mg/kg, IP) and in combination with methamphetamine (1.5 mg/kg, IP) on the locomotor activity of male mice.

**: P<0.01, as compared to saline control.
***: P<0.001, as compared to saline control.
#: P<0.05, as compared to saline + methamphetamine group.
##: P<0.01, as compared to saline + methamphetamine group.
###: P<0.001, as compared to saline + methamphetamine group.

Figure 4 - Effect of methysergide alone (1 mg/kg, IP) and in combination with methamphetamine (1.5 mg/kg, IP) on the locomotor activity of male mice.

**: P<0.01, as compared to saline control.
***: P<0.001, as compared to saline control.
##: P<0.01, as compared to saline + methamphetamine group.
###: P<0.001, as compared to saline + methamphetamine group.

Figure 5 - Effect of methamphetamine (3 mg/kg, IP) alone and methysergide (1 mg/kg, IP) in combination with methamphetamine (3 mg/kg, IP) on the locomotor activity of male mice.

Figure 6 - Effect of mianserin alone (8 mg/kg, IP) and in combination with methamphetamine (1.5 mg/kg, IP) on the locomotor activity of male mice.

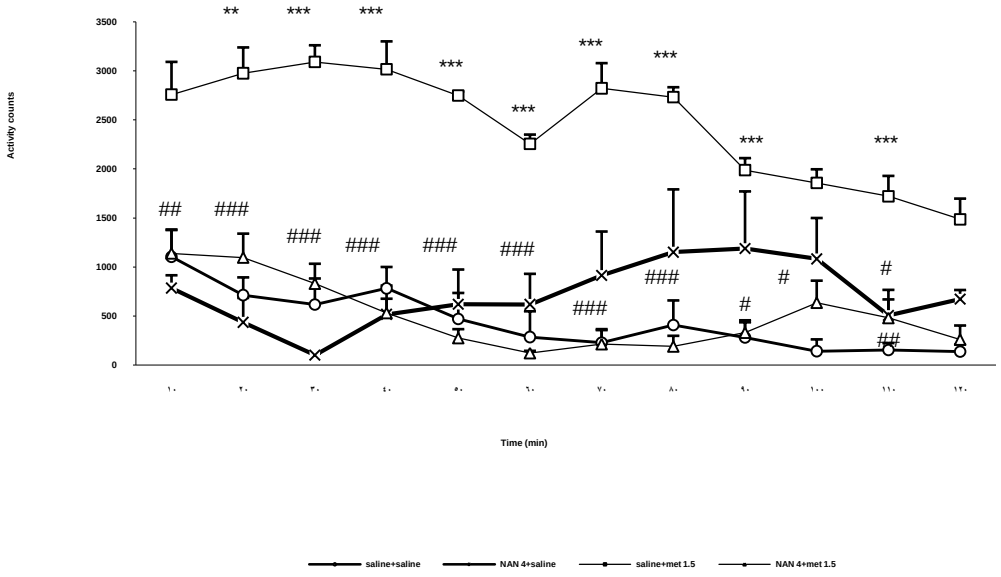
**: P< 0.01, as compared to saline control.
***: P< 0.001, as compared to saline control.
#: P< 0.05, as compared to saline + methamphetamine group.
##: P< 0.01, as compared to saline + methamphetamine group.
###: P< 0.001, as compared to saline + methamphetamine group.

Figure 7 - Effect of methamphetamine (3 mg/kg, IP) alone and mianserin (8 mg/kg, IP) in combination with methamphetamine (3 mg/kg, IP) on the locomotor activity of male mice.

Figure 8 - Effect of ondansetron alone (0.5 mg/kg, IP) and in combination with methamphetamine (1.5 mg/kg, IP) on the locomotor activity of male mice.

**: P< 0.01, as compared to saline control.
***: P< 0.001, as compared to saline control.
##: P< 0.01, as compared to saline + methamphetamine group.
###: P< 0.001, as compared to saline + methamphetamine group.

Figure 9 - Effect of methamphetamine (3 mg/kg, IP) alone and ondansetron (0.5 mg/kg, IP). in combination with methamphetamine (3 mg/kg, IP) on the locomotor activity of male mice.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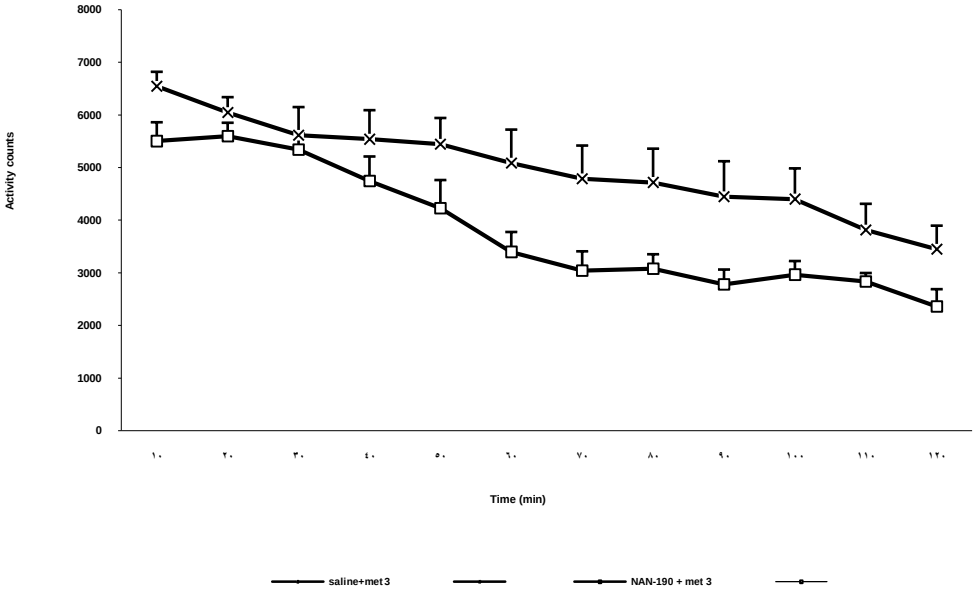
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Time (min)
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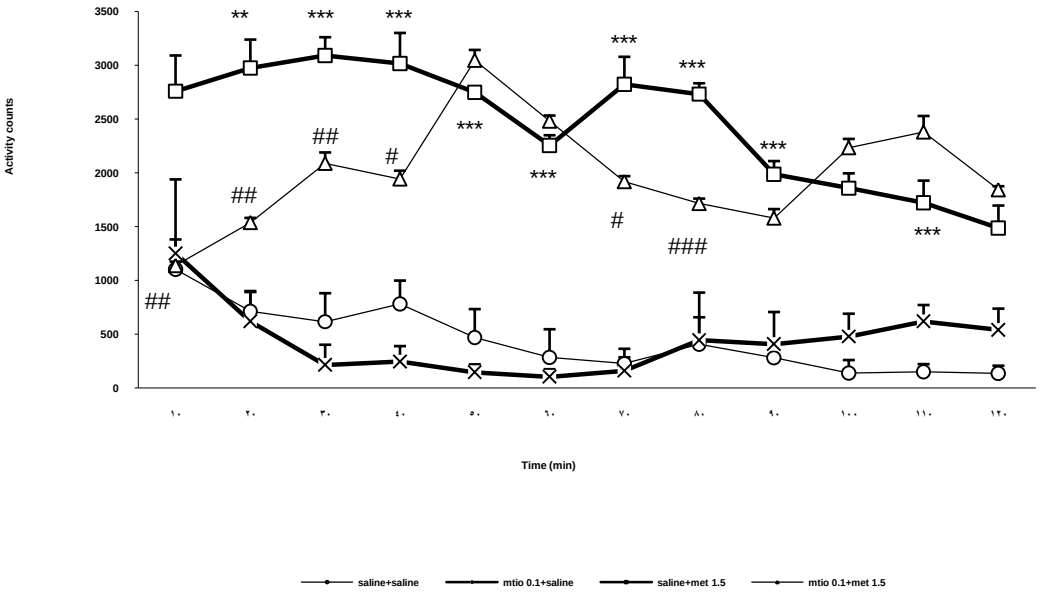
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Time (min)
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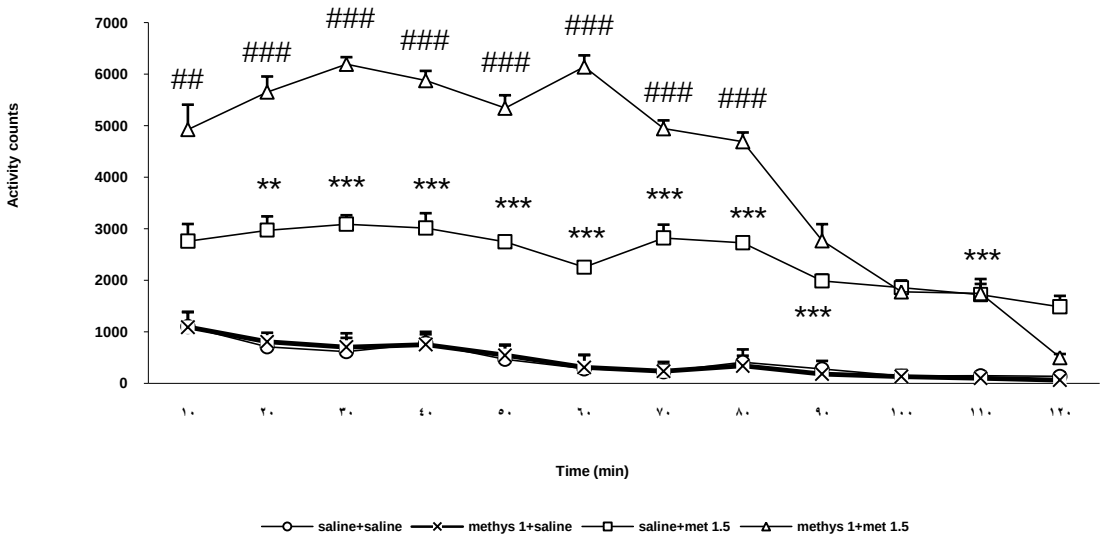
saline+met 1.5
mtio 0.1+met 1.5


Activity counts
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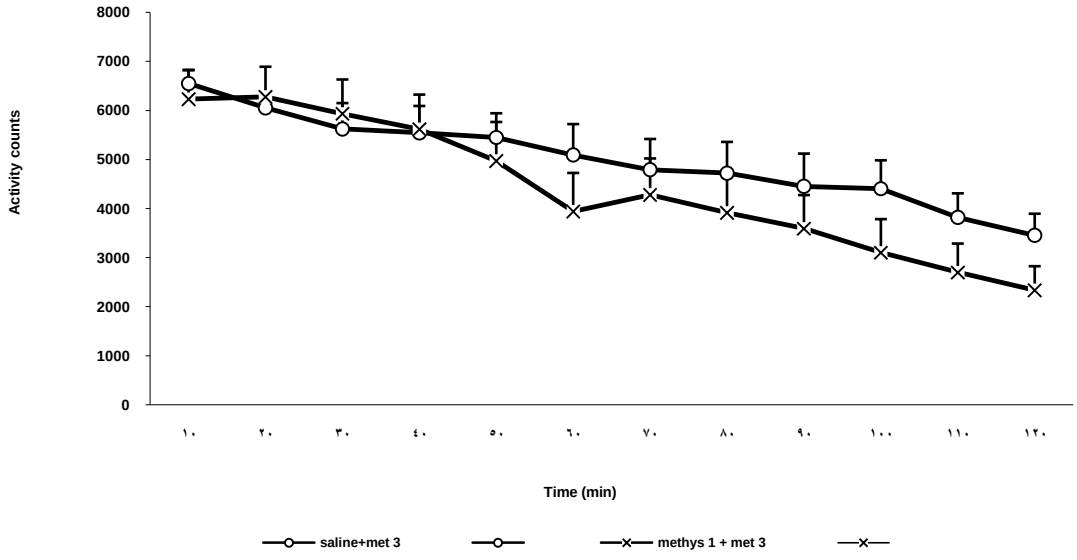
Time (min)
saline+saline
methys 1+saline
saline+met 1.5
methys 1+met 1.5


8000
7000
6000
5000
4000
3000
2000
1000
0
Activity counts
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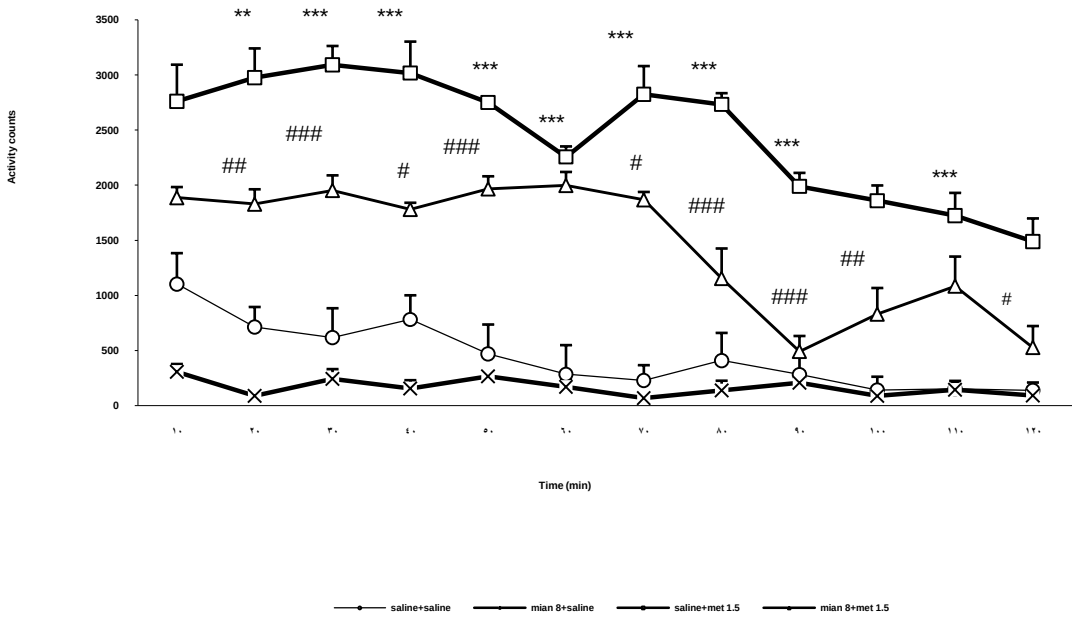
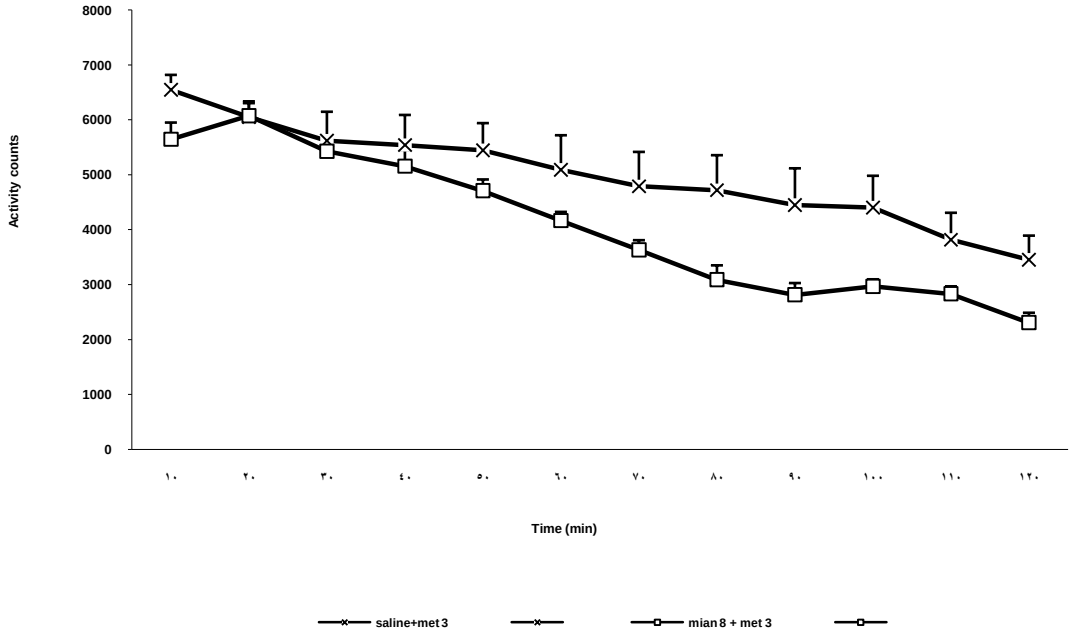
Time (min)
saline+met 3
methys 1 + met 3


Activity counts
3500
3000
2500
2000
1500
1000
500
0
Time (min)
saline+saline
mian 8+saline
saline+met 1.5
mian 8+met 1.5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Activity counts
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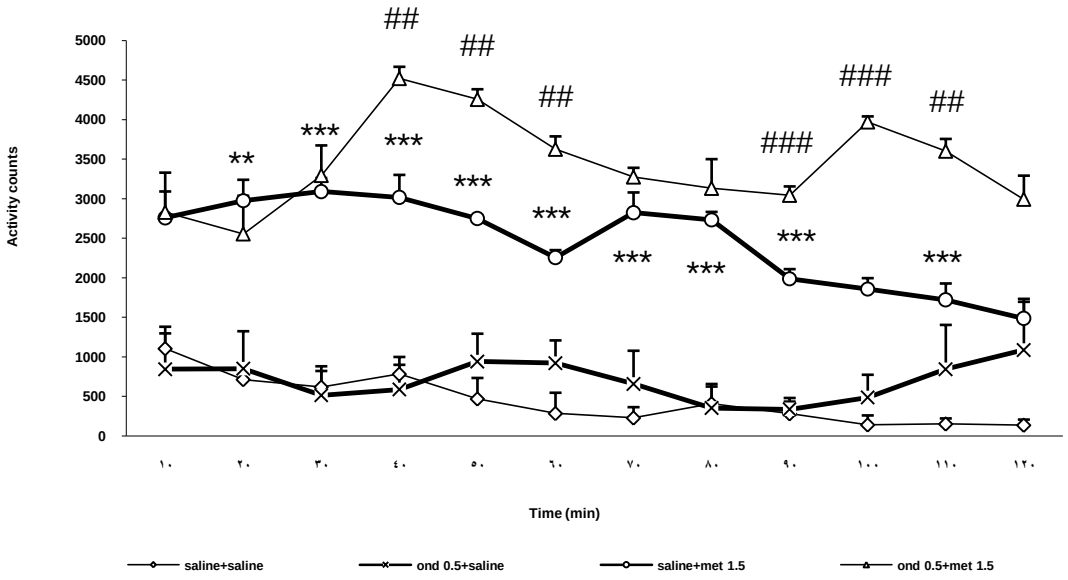
Time (min)
saline+met 3
mian 8 + met 3


Activity counts
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3500
3000
2500
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1000
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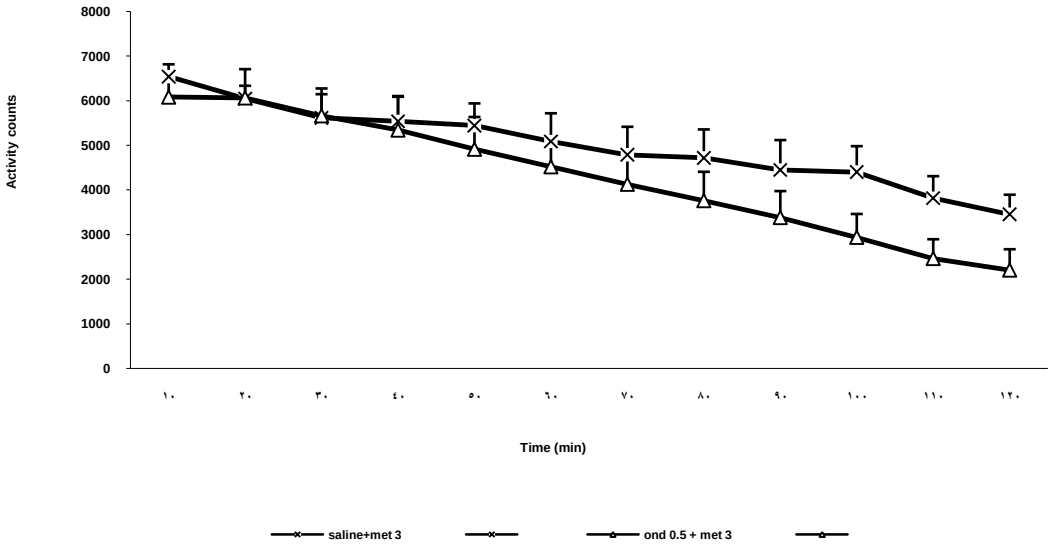
Time (min)
saline+saline
ond 0.5+saline
saline+met 1.5
ond 0.5+met 1.5


8000
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Activity counts
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Time (min)
saline+met 3
ond 0.5 + met 3