Effects of sertraline on experimental mouse models of psychosis

Ugur Bilge, MD, Kevser Erol, PhD.

ABSTRACT

الأهداف: دراسة تأثير عقار السيرترالين على التجمد المُحفز بمضادات الذهان، والسلوكيات المتصاعدة المُحفزة بالأبوموفرين، والأنشطة الحركية المُحفزة بالأمفيتامين أو MK-801 وذلك لدى فئران الألبينو الإناث اللاتي تتراوح أوزانهن ما بين 30-35 غرام.

الطريقة: أُجريت هذه الدراسة في قسم الصيدلة، جامعة إيسكير أوزمانغازي، إيسكير، تركيا، واستمرت خلال الفترة من أبريل 2008م إلى يناير 2010م. لقد قمنا بتحفيز التجمد باستخدام هالوبيرودول (1 ملغ/كلغ داخل الصفاق)، فيما قمنا بحقن أبوموفين (1.5 ملغ/كلغ تحت الجلد) من أجل دراسة السلوكيات المتصاعدة. وقمنا بتحفيز الأنشطة الحركية بواسطة حقن دي أمفيتامين (30 ملغ/كلغ داخل الصفاق)، أو (0.3 ملغ/كلغ داخل الصفاق) من MK-801. لقد قمنا بتوزيع 8 فئران في كل مجموعة حيث قمنا بحقن عقار السيرترالين بحدة (10 ملغ/كلغ داخل الصفاق) أو على مدى 5 أيام بجرعات متكررة.

النتائج: أشارت نتائج الدراسة إلى أن عقار السيرترالين قد قام بمنع الإصابة بالتجمد والسلوكيات المتصاعدة وذلك عندما قمنا بحقنه على مدى 5 أيام وبجرعات متكررة. بالمقابل فقد زاد هذا العقار الأنشطة الحركية المُحفزة بالأمفيتامين. ولقد قام هذا العقار بتقليل الحركات النمطية المُحفزة بحقن MK-281، غير أنه لم يؤثر تأثيراً واضحاً من الناحية الإحصائية على الحركات النمطية المحفزة بالأمفيتامين وذلك عند استخدامه بجرعات منفردة أو متكررة.

خاتمة: أظهرت النتائج بأن السيرترالين الذي يعد من مثبطات استرداد السيرتونين الإنتقائية قد يكون عقاراً مساعداً أثناء علاج مرض الذهان.

Objective: To study the effects of sertraline on neuroleptic-induced catalepsy, apomorphine-induced climbing behavior, and amphetamine or MK-801-induced locomotor activities in female Swiss albino mice weighing 30-35 g.

Methods: This study was performed in the Department of Pharmacology, Eskisehir Osmangazi University, Eskisehir, Turkey between April 2008 and January 2010. Catalepsy was induced by haloperidol (1 mg/kg intraperitoneally [ip]). Apomorphine (1.5 mg/kg subcutaneously [sc]) was used for studying climbing behavior, and d-amphetamine (30 mg/kg ip) or MK-801 (0.3 mg/kg ip) was used for testing locomotor activities. Eight animals were used in each group. Sertraline (10 mg/kg ip) was injected either acutely, or over 5 days of repeated treatment.

Results: Sertraline inhibited catalepsy and climbing behavior when it was used for 5 days in repeated doses, while it augmented amphetamine-induced locomotor activity. It reduced MK-801-induced stereotypic movements, but did not significantly affect amphetamine-induced stereotypic movements when used in a single dose or repeated doses.

Conclusion: These results suggest that sertraline, a selective serotonin reuptake inhibitor may be a beneficial adjuvant drug during psychosis therapy.

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From the Department of Pharmacology, School of Medicine, Eskisehir Osmangazi University, Eskisehir, Turkey.

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Address correspondence and reprint request to: Prof. Dr. Kevser Erol, Department of Pharmacology, School of Medicine, Eskisehir Osmangazi University, Eskisehir 26480, Turkey. Tel. +90 (222) 2392979. Fax. +90 (222) 2393772. E-mail: kerol@ogu.edu.tr

Recently, it has been reported that treating negative symptoms of schizophrenia with a combination of a typical antipsychotic and a selective serotonin reuptake inhibitor (SSRI) was more effective than treatment with antipsychotics alone.[1] The molecular mechanisms underlying this augmentation are unclear. Several SSRIs inhibit cytochrome P450 (CYP) enzymes, mainly CYP1A2 and CYP2D6 isoenzymes, which are metabolizers of antipsychotic drugs.[2,3]

Therefore, higher plasma levels of antipsychotic drugs are frequently expected with the combination of SSRIs and antipsychotics.[4] It was shown that the improvement of negative symptoms in schizophrenic patients using the combinations of SSRI with haloperidol did not correlate with the increase in plasma levels of the antipsychotic.[5] However, several effective augmentation treatments for schizophrenia used SSRIs that have no pharmacokinetic interaction with antipsychotics.[4,6] Finally, pharmacokinetic interactions cannot explain the clinical therapeutic augmentations for negative symptoms of psychosis. It was suggested that the effects of SSRIs, as augmentation agents on negative symptoms of psychosis, appear to be distinct from non-specific antidepressant action. Atypical antipsychotics such as clozapine, which may improve negative symptoms, enhance dopamine efflux in the frontal cortex while typical drugs such as haloperidol, ineffective against negative symptoms do not induce dopamine release.[7] Sertraline is one of the most used SSRIs. It is known that it has dopamine stimulating and dopamine-reuptake inhibitory effects in addition to its serotonergic activity.[8] With regard to the effects of sertraline on the serotonergic and dopaminergic system, we wanted to evaluate the effects of sertraline on hyperactivity induced by systemic administration of the N-Methyl-D-aspartic acid (NMDA) receptor antagonist MK-801, and the indirect dopamine agonist amphetamine as 2 pharmacological models of schizophrenia and test its ability to induce catalepsy in mice as a model for extrapyramidal side-effects. The present study was designed to evaluate the possible antipsychotic-like profile of sertraline itself when used as a single dose or a repeated dose schedule in mice. Amphetamine or MK-801-induced locomotor activities, neuroleptic-induced catalepsy and apomorphine-induced climbing behavior test systems were used for determining the antipsychotic-like activities of sertraline.

Methods. This study was performed in the Department of Pharmacology, Eskisehir Osmangazi University, Eskisehir, Turkey between April 2008 and January 2010. Female Swiss albino mice, up to 3-4 months old (weighing 30-35 g), were used in the study. The animals were maintained in our own animal facilities under a controlled environment (21±2°C 12 hour light/dark cycle, with free access to standard food and water). All behavioral experiments were in accordance with the Guidelines for Animal Care of Eskisehir Osmangazi University, and institutional ethical approval to conduct this study was obtained. Different groups of animals were used in the separate experiments. Six or 8 animals were used in each group. All experiments were conducted between the hours of 9.00 and 13.00.

Drugs and groups. Sertraline HCl (Sanovel, Istanbul, Turkey), Haloperidol ampule (Ali Raif, Istanbul, Turkey), MK 801 and d-amphetamine sulphate (Sigma-Aldrich, St Louis, MO, USA), and apomorphine HCl ampule (Gen, Istanbul, Turkey) were used in the study. The animals were randomly divided into 4 groups. Groups one, 3, and 4 were divided into 3 subgroups. Locomotor activity test, catalepsy, and climbing tests were applied to each subgroup. Group 2 was divided into 2 subgroups. Only locomotor activity tests were applied after treatment by amphetamine or MK-801 to these subgroups. Eight animals were used for the locomotor activity test, and 6 animals were used for the catalepsy and climbing tests. Group one was the control, and was treated with saline for 5 days. Locomotor activity, climbing, and catalepsy tests were applied. Group 2 was the amphetamine or MK-801 group, and was treated with saline for 5 days. On the last day the animals received amphetamine or MK-801. Only locomotor activity tests were applied. Group 3 was the acute sertraline group, which received intraperitoneal (ip) saline for 4 days, and 10 mg/kg sertraline on the fifth day. Locomotor activity, climbing, and catalepsy tests were applied. Group 4 was the chronic sertraline group, which received ip 10 mg/kg sertraline for 5 days. Locomotor activity, climbing, and catalepsy tests were applied.

Locomotor activity. On the fifth day, one hour after the last dose of sertraline or saline, mice were treated intraperitoneally with 3 mg/kg amphetamine,[9] or 0.3 mg/kg MK-801.[10] Five minutes later, spontaneous locomotor activity was recorded for 5 minutes in every half hour over 3 hours by using an animal activity monitoring system (MAY Activity Monitoring System 02; Commat, Ankara, Turkey). Animals had not been previously habituated to the boxes. To assess locomotor activity, mice were placed into a square Plexiglas box (40 x 40 x 40 cm). Locomotor activity was recorded by a video-computerized system. The software tracked the animals by distinguishing their white color from the black background of the floor registering X and Y horizontal coordinates. The method was set to examine stereotypic, horizontal, ambulatory, and total movements, as well as traveled distance, ignoring small movements such as breathing and tremors. The results of each activity were recorded automatically by a video-computerized system.

Catalepsy. After treating the mice with either drug or saline, catalepsy was induced by haloperidol (1.5 mg/kg subcutaneously [sc]),[10] as a model for extrapyramidal side-effects. One hour after the injection of haloperidol, catalepsy time was determined for 5 minutes in every hour over 3 hours. Catalepsy time was measured after mice forepaws were placed over a horizontal glass bar (0.6 cm diameter) elevated 6 cm from the floor. The

time that mice maintained both forepaws on the ground was recorded allowing 3 immediate attempts to replace the animal in the cataleptic position within the first 10 seconds. Mice that did not move their paws, but showed active body or head movements were not considered as cataleptic.

Apomorphine-induced climbing. Apomorphine (15 mg/kg sc) was used for studying climbing behavior.[10] Saline or sertraline was injected one hour before the administration of apomorphine. Immediately after the injection of apomorphine, mice were placed individually in cylindrical wire mesh cages (height 13 cm, diameter 14 cm, mesh size 3 mm). During the trial the total time when each mouse climbed on the inside of the wire cage was recorded over 30 minutes.

Statistical analysis. Using the Statistical Package for Social Sciences version 15 (SPSS Inc., Chicago, IL, USA) the data were expressed as mean ± SEM and results were analyzed using 2-way analysis of variance (ANOVA) with time as the repeated measure and Student's t test. A value of $p<0.05$ was considered statistically significant.

Results. ***Locomotor activity.*** Single and repeated doses of sertraline enhanced time dependently amphetamine-induced ambulatory movements [$F_{(3,20)}=53.965$; $p<0.001$] (**Figure 1a**). Repeated doses of sertraline increased horizontal movements [$F_{(3,20)}=7.196$; $p<0.002$] (**Figure 1b**), distances [$F_{(3,20)}=14.718$; $p<0.001$] (**Figure 1c**), and total movements [$F_{(3,20)}=30.955$; $p<0.001$] (**Figure 1d**) induced by pretreatment with amphetamine. The maximum effects of amphetamine were observed at the sixtieth minute and decreased gradually on ambulatory, horizontal, total movements, and distance. Both administrations of sertraline prevented these effects of amphetamine for 3 hours. Locomotor activity was evaluated by analyzing the ambulatory, total movements, and distance traveled. Horizontal activity was analyzed as the primary index of exploration. The effects of sertraline on ambulatory, horizontal, total movements, and distance induced by amphetamine when using repeated doses were higher than a single dose (**Figures 1a, b, c, d**). The stereotypic behavior reduced time-dependently and was suppressed by amphetamine [F(3.20=7.919; $p<0.001$]. Single and repeated doses of sertraline did not significantly change amphetamine-induced stereotypy (**Figure 1e**), while single and repeated doses of sertraline significantly suppressed stereotypic movements - induced by

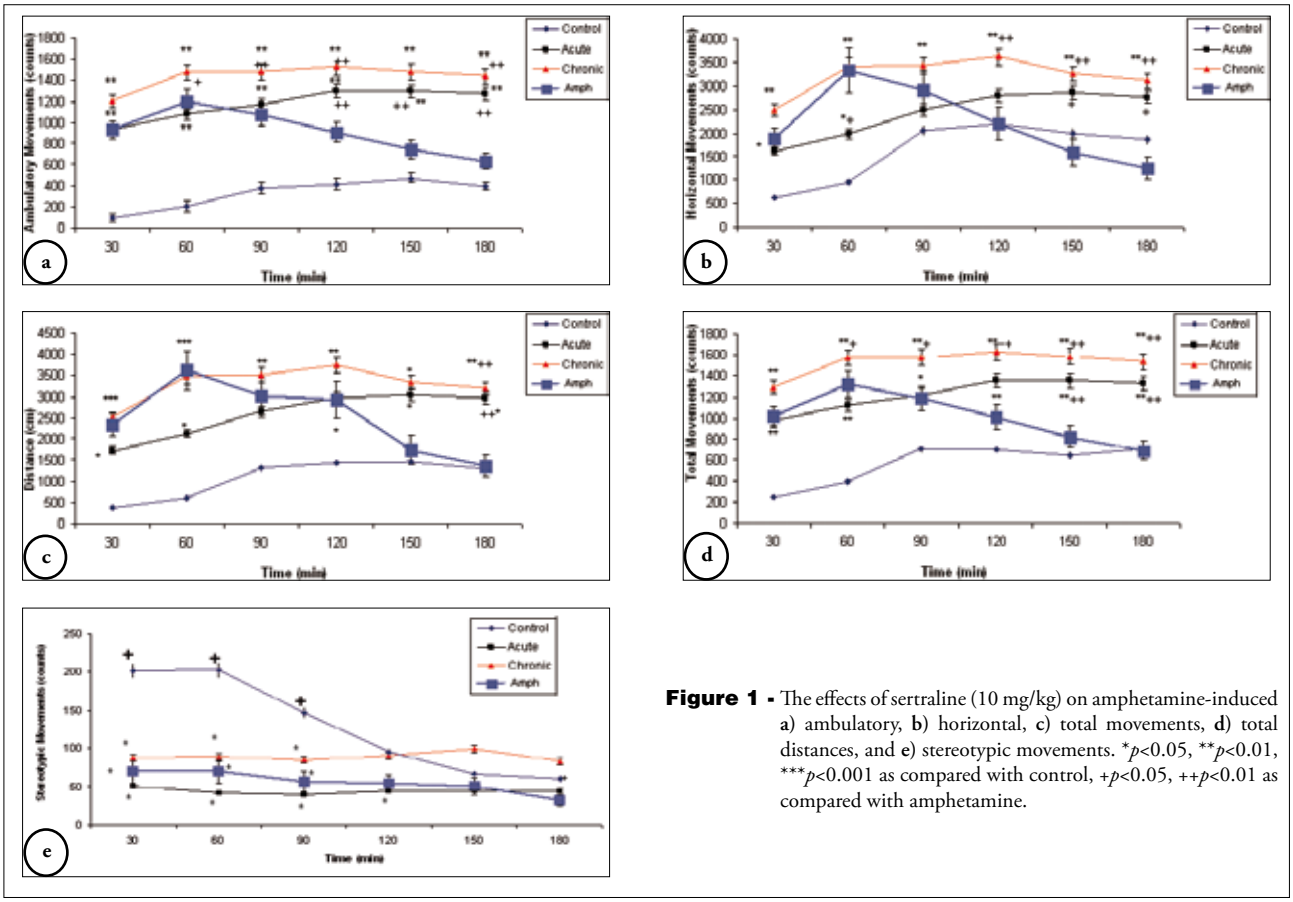

Figure 1 - The effects of sertraline (10 mg/kg) on amphetamine-induced **a)** ambulatory, **b)** horizontal, **c)** total movements, **d)** total distances, and **e)** stereotypic movements. *$p<0.05$, **$p<0.01$, ***$p<0.001$ as compared with control, +$p<0.05$, ++$p<0.01$ as compared with amphetamine.

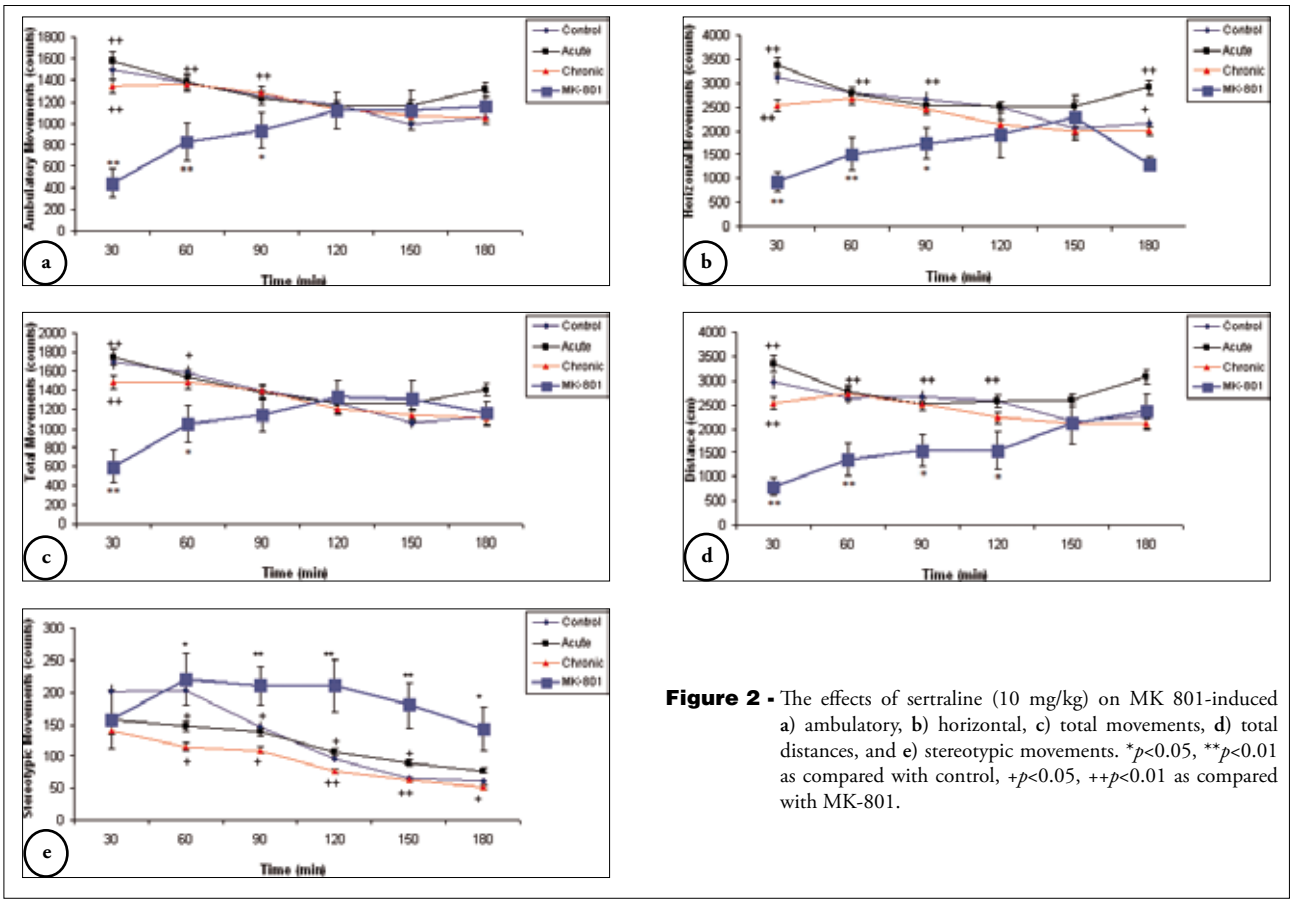

Figure 2 - The effects of sertraline (10 mg/kg) on MK 801-induced **a**) ambulatory, **b**) horizontal, **c**) total movements, **d**) total distances, and **e**) stereotypic movements. *p<0.05, **p<0.01 as compared with control, +p<0.05, ++p<0.01 as compared with MK-801.

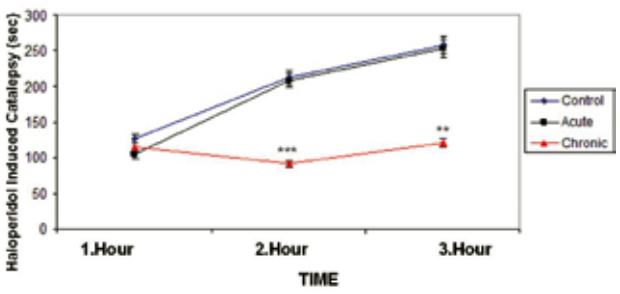

Figure 3 - Chronic sertraline attenuated catalepsy induced by haloperidol (1 mg/kg i.p.). **p<0.01, ***p<0.001 as compared with control and acute sertraline

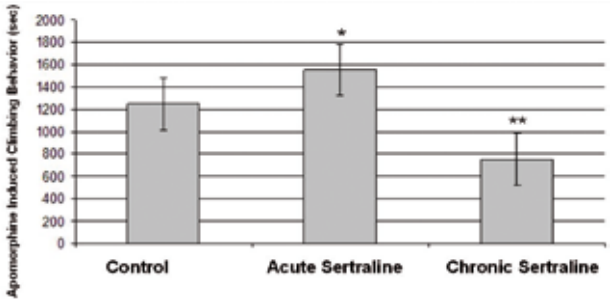

Figure 4 - Chronic sertraline attenuated while acute sertraline augmented climbing behavior induced by the dopamine agonist apomorphine (1.5 mg/kg s.c.). *p<0.05, as compared with control.

pretreatment with MK-801 [F(3.20=5.176; p<0.008] (**Figure 2e**). Stereotypic behavior was evaluated as one of the symptoms of psychosis. The MK-801 decreased ambulatory (**Figure 2a**), horizontal (**Figure 2b**), and total movements (**Figure 2c**), as well as distance (**Figure 2d**) during the first hours, and then they increased gradually. Single dose or repeated doses of sertraline increased these movements and there were no significant changes between control and sertraline groups (**Figures 2 a, b, c, d**).

Catalepsy. Sertraline inhibited catalepsy induced by haloperidol (**Figure 3**) and climbing behavior induced by apomorphine when it was used for 5 days in repeated doses (**Figure 4**). A single dose of sertraline did not change the cataleptogenic effects of haloperidol (**Figure 3**). Neuroleptic induced catalepsy is thought to reflect the extrapyramidal side effects of antipsychotic agents and is used as a pre-clinical test for screening new antipsychotics.

Climbing. A single dose of sertraline slightly increased climbing behavior, but repeated doses of sertraline decreased it significantly (**Figure 4**). These results suggest that SSRI's may have beneficial effects against extrapyramidal side effects of antipsychotic drugs during the treatment of psychosis. An antipsychotic drug is expected to inhibit apomorphine-induced climbing behavior.

Discussion. The present study evaluated the effects of sertraline at single or repeated doses on amphetamine-, or MK-801-, induced locomotor activity, neuroleptic-induced catalepsy, and apomorphine-induced climbing behavior in mice. Sertraline especially augmented amphetamine-induced locomotor activity. But its effects on MK-801-induced locomotor activity were not as high as those of amphetamine. A single dose of sertraline did not change haloperidol-induced catalepsy, but its administration in repeated doses significantly decreased the cataleptic effect of haloperidol and apomorphine-induced climbing behavior. With regard to the results of locomotor activities, amphetamine increased ambulatory, horizontal, and total movements in the first hour, and then decreased gradually to nearly basal levels after 90-120 minutes, while MK-801 suppressed these movements for the first 2 hours. Sertraline augmented the effects of amphetamine and recovered the effects of MK-801.

Glutamate NMDA receptor antagonists have been regarded as a pharmacological model for psychosis, by producing hyperlocomotion and cognitive deficits in rodents.[11] Typical antipsychotics are known to inhibit typically hyperactivity induced by NMDA receptor antagonists only at doses that inhibit spontaneous activity.[12] The MK-801, a high affinity analogue of phencyclidine, is a noncompetitive NMDA receptor antagonist that binds to the hydrophobic domain of this ligand-gated channel. Phencyclidine is known to precipitate a schizophreniform psychosis, mimicking symptoms in psychopathology, including positive (for example, hallucinations), negative (for example, affective flattening, amotivation, and social withdrawal) and cognitive (for example, concretization of thought and impaired ability to abstract).[12] It was reported that behaviors elicited by MK-801 reflect a pharmacologically–induced state of NMDA receptor hypofunction, which has been proposed to exist in schizophrenia.[13] Our results suggest that sertraline could be effective on NMDA receptor hypofunction in psychosis.

Amphetamine-induced hyperlocomotion has long been used as a pharmacological model for psychosis in animals,[14] and is most useful in preclinical research for new antipsychotic drugs, as it may mimic the hyperdopaminergic tone present in many schizophrenic patients.[15] Acute administration of SSRIs caused transient decreases, while repeated administration of these drugs may result in increased dopamine function and upregulation in dopamine function.[8] The effects of SSRI augmentation on dopaminergic neurotransmission appears to involve selective changes in activation of 5HT receptors throughout the brain. It was reported that sertraline facilitates dopaminergic neurotransmission, and this dopaminergic activity clearly distinguishes it from all other SSRIs that do not have such activity.[8] Sertraline augmented amphetamine-induced hyperlocomotion, especially when used in repeated dose of 10 mg/kg in the present study. At least part of this effect should result from the dopaminergic activity of sertraline. A single dose of sertraline (10 mg/kg sc) alone was shown to increase 5-HT and norepinephrine in the rat prefrontal cortex, but not dopamine.[16] The malfunction of prefrontal cortex is known from the cognitive and emotional defects in affective and psychiatric disorders such as depression and schizophrenia. The increase in striatal dopamine concentration was observed following sertraline administration.[17] The SSRIs were demonstrated to enhance locomotor activity.[18] The augmentation of amphetamine-induced locomotor activity in our results may be related to the increasing effects of sertraline on central dopaminergic activity.

Other interesting results of the present study were that sertraline attenuated MK-801-induced stereotypic behavior, while it did not significantly change amphetamine-induced stereotypic behavior. It is known that both NMDA antagonists and agents that increase, or mimic dopamine, increase locomotion and induce stereotypic behavior. However, the 2 classes of drugs have different effects. Stereotypy has been conceptualized as the selective increase in the amounts of certain types of behaviors (which are performed repetitively). These selective behaviors change as the dose is increased, and the induced locomotion also has a repetitive nature compared with normal locomotion.[19,20] The stereotypic behavior induced by MK-801 is also distinct from that of amphetamine. It was reported that MK-801 has bimodal effects on locomotion and stereotypy, and the hyperlocomotion phase develops only after the stereotypic phase subsides at high doses (above 0.3 mg/kg).[20] Our results of MK-801-induced stereotypy and locomotion are similar to these findings. So it was thought that MK-801 suppressed the locomotion at this dose level (0.3 mg/kg). Recently, it was suggested that NMDA antagonists-induced hyperlocomotion can occur independent of nigrostriatal dopamine circuits.[21,22]

It was reported that some psychoactive drugs including haloperidol, methamphetamine, fluvoxamine and sertraline have affinity for σ1 receptors.[23] These receptors play a modulatory role in the activity of some ion channels and several neurotransmitter systems, mainly glutamatergic neurotransmission. Sigma1 receptors were thought to be able to play a role in the pathogenesis of schizophrenia. The antagonists of σ1 receptors were reported to have antipsychotic activity in animal models predictive of efficacy in schizophrenia.[23,24] It is accepted that σ receptor ligands may play a role in dopaminergic neurotransmission. It is suggested that the antagonists of σ receptors attenuated apomorphine-induced climbing,[24] but did not induce catalepsy.[25] We also observed that sertraline reduced apomorphine-induced climbing and haloperidol-induced catalepsy when administered repeatedly. These results suggested that sertraline should act on σ receptors.

Haloperidol-induced catalepsy is known to be a robust behavioral model for evaluating nigrostriatal function. This characteristic catalepsy response in rodents is thought to be more dependent upon striatal dopamine than mesolimbic dopamine circuits. It is accepted that the drugs that are able to attenuate catalepsy in rodents might reduce the extrapyramidal signs of Parkinson disease. It was shown that haloperidol-induced catalepsy was attenuated by 5-HT1A agonists.[26,27] It was reported that SSRIs activate 5HT-1A receptors, antagonize 5-HT2 heteroreceptors, mainly 5-HT2A and 5-HT2C when used with antipsychotics.[28] We observed that sertraline attenuated haloperidol induced catalepsy only when used in repeated doses. There was no significant difference when sertraline was used in a single dose. Our results contradict the results reported that acute sertraline attenuated dose-dependent haloperidol-induced catalepsy (at 180 minutes after haloperidol).[29] The cut off time in that study was 720 seconds, while ours was 300 seconds. This discrepancy might be related to the animal strain or laboratory conditions. The anticataleptogenic effect of sertraline used in repeated doses, might be speculated, to be related to activation of 5-HT1A receptors.[26,27,29] However, the attenuation of haloperidol-induced catalepsy could be beneficial to reduce the extrapyramidal side effects of antipsychotic drugs.

Apomorphine is a dopamine agonist and exhibits climbing behavior. It was suggested that postsynaptic mesolimbic D1 and D2 receptors may mediate climbing behaviour.[30] We also found that sertraline insignificantly augmented apomorphine-induced climbing behavior when used in a single dose and significantly attenuated it in repeated doses. These results suggest that repeated doses of sertraline might decrease dopaminergic activity or desensitize mesolimbic dopaminergic receptors.

In conclusion, experimental psychosis is a very complicated area. Much research is needed to identify the molecular mechanisms of SSRI-antipsychotic treatments. The main finding of this study is that sertraline augmented amphetamine-induced locomotor activity and attenuated MK-801-induced stereotypy, apomorphine-induced climbing and haloperidol-induced catalepsy when used in repeated doses. These results suggest that sertraline might be a putative adjuvant drug during antipsychotic therapy. However, the possibility that it increases locomotor activity should also be remembered. It is necessary to determine the putative profile of sertraline as a long-lasting adjuvant drug by using different doses, periods, and experimental methods such as the prepulse inhibition test. The present data confirm that sertraline could have antipsychotic activity and exhibits low extrapyramidal symptoms by affecting serotonergic and dopaminergic systems. Since psychosis has various and complex characteristics, it is very important to evaluate the validity of the animal models limited to each feature of psychosis, and to choose a suitable model for determining the novel therapeutic targets.

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STATISTICS

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Describe statistical methods with enough detail to enable a knowledgeable reader with access to the original data to verify the reported results. When possible, quantify findings and present them with appropriate indicators of measurement error or uncertainty (such as confidence intervals). Avoid relying solely on statistical hypothesis testing, such as the use of *P* values, which fails to convey important information about effect size. References for the design of the study and statistical methods should be to standard works when possible (with pages stated). Define statistical terms, abbreviations, and most symbols. Specify the computer software used.