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ADENOSINE A_{2A} RECEPTOR ANTAGONISM INCREASED THE ANTIDEPRESSANT-LIKE EFFECT OF AMITRIPTYLINE IN MICE
AMİTRİPTİLİNİN FAREDEKİ ANTİDEPRESAN BENZERİ ETKİSİNİN ADENOZİN A_{2A} RESEPTÖR ANTAGONİZMASI İLE ARTIŞI

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ABSTRACT

Objective: The role of the endogenous adenosinergic system in the peripheral effect of an antidepressant drug, amitriptyline, has been demonstrated in pain models and also in cardiovascular toxicity induced by amitriptyline. We performed this study as there is no information on whether adenosine or adenosine A2A receptor antagonists have any effect on the antidepressant-like activity of amitriptyline in mice.

Methods: Balb-c mice were used in experiments and forced swimming test was used to evaluate the antidepressant-like activity. Mice were injected with saline (control), amitriptyline, SCH 58261 (A_{2A} receptor antagonist), SCH 58261 + amitriptyline, adenosine, adenosine + amitriptyline, intraperitoneally.

Results: Amitriptyline decreased immobility time compared to control group at both doses. SCH 58261 did not produce antidepressant like effect in the applied dose alone. Pretreatment of amitriptyline with SCH 58261 produced stronger inhibition of immobility time than amitriptyline induced alone in the same dose. Co-administration of adenosine with amitriptyine, however, it was not decreased the anti-immobility effect of amitriptyline. However, it was not statistically significant.

Conclusion: It has been demonstrated that adenosinergic system may have a role in the antidepressant-like activity of amitriptyline in mice, and pretreatment with the A_{2A} receptor antagonist may induce a more pronounced antidepressant-like activity. We are of the opinion that this finding may be of particular importance in the case of drug resistant patients.

Keywords: Amitriptyline, adenosine, adenosine A2A receptor, antidepressan activity, mouse

ÖZ

Amaç: Antidepresan bir ilaç olan amitriptilinin periferal etkilerinde adenozinerjik sistemin rolü olduğu ağrı modellerinde ve amitriptilinin neden olduğu kardiyovasküler sistem toksisitesinde gösterilmiştir. Ancak amitripitilinin antidepresan aktivitesinde adenozin ya da adenozin A_{2A} reseptör antagonismasının rolü olup olmadığına dair bilgi bulunmadığı için bu çalışmayı planladık.

Metod: Deneylerde antidepresan benzeri etkiyi tayin için Balb-c fareler üzerinde zorunlu yüzme testi uygulanmıştır. Farelere serum fizyolojik (kontrol grubu), amitriptilin, SCH 58261 (A_{2A} reseptör antagonisti), SCH 58261+amitriptilin, adenozin, adenozin+amitriptilin intraperitoneal yoldan uygulanmıştır.

Bulgular: Amitriptilin her iki dozunda da kontrol grubuna göre hareketsiz kalma zamanını azaltmıştır. Amitriptilinin hareketsiz kalma süresini azaltıcı etkisi SCH 58261 ile ön muamele edildiğinde daha da artmıştır. Adenozin ile amitriptilin birlikte uygulandığında ise amitriptilinin hareketsiz kalma süresini azaltıcı etkisi azalmakla birlikte bu etki istatistiksel olarak anlamlı bulunmamıştır.

Tartışma: Amitriptilinin faredeki antidepresan benzeri etkisinde adenozinerjik sistemin rolü olabileceği ve A_{2A} receptor antagonisti ile ön muamele edildiğinde bu etkinin artabileceği gösterilmiştir. Bu bulgunun özellikle ilaca dirençli hastalarda önemli olabileceğini düşünmekteyiz.

Anahtar kelimeler: Amitriptilin, adenozin, adenozin A_{2A} reseptör, antidepresan etki, fare

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INTRODUCTION

Major depressive disorder (MDD) is a chronic and often life-threatening common mental illness (1). Amitriptyline is a frequently prescribed tricyclic antidepressant (TCA) drug that was developed for the treatment of MDD but also used to treat depression, panic attacks, anxiety disorder, and bulimia nervosa. Amitriptyline shows its antidepressant activity by inhibiting neuronal reuptake of both serotonin and noradrenaline. At the same time it also has antagonistic actions at α1-adrenergic, histamine, muscarinic and serotonine receptors, and blocks sodium, calcium and potassium channels. Due to this multicipty of action, amitriptyline cause side effects such as constipation, sedation, blurred vision and cardiovasculer toxicities (2 -4). At the same time TCA drugs are also effective in chronic pain states such as fibromyalgia and neuropathy (5-7). It has been shown that TCAs inhibit the neuronal adenosine uptake in the peripheral and central nervous system (CNS) levels and produce antinociception (8-10).

A purine nucleoside adenosine acts as a neuromodulator in the CNS [11] and exerts its actions at four different receptors; adenosine A_1, A_{2A}, A_{2B} and A_3 receptors (12,13). A_1 and A_{2A} receptors are abundantly found in the CNS and basal adenosine levels can activate them (14,15). Endogenous adenosine is involved in the regulation of cognition, memory, arousal, aggression and anxiety (11,15,16). However, the results of studies concerning the role of adenosine in depression is highly controversial. In some animal studies administration of adenosine and its analogues have been shown to produce depressant-like effects (17,18). Additionally, it was shown that the adenosine A_{2A} receptor antagonists produce an antidepressant-like effect in mice and A_{2A} receptor knockout animals have less immobility time in forced swimming test (FST), tail suspension test (TST) (19-23), and in reserpine induced depression model (24). Chronic unpredictable stress (CUS) induced mood and synaptic dysfunction was reversed by the selective A_{2A} receptor antagonist and also in A_{2A} receptor knockout mice (25). In contrast, some other studies have shown that adenosine and adenosine receptor agonists produces an antidepressant-like effect in the FST in mice which is mediated through an interaction with A_1 and A_{2A} receptors (26-28). As it was shown that TCA drugs act as a neuronal adenosine uptake inhibitor (7-10), can bind to adenosine receptors (29,30), and reduce the activity of ecto-nucleotidases (31), we performed this study to investigate whether adenosine or adenosine A_{2A} receptor antagonist have any effect on the antidepressant-like activity of amitriptyline in mice.

MATERIAL AND METHODS

Experiments were conducted in accordance with relevant ethical guidelines and approved by the Erciyes University Local Ethics Committee for Animal Experiments. The experiment was carried out on either sex of Balb-c mice, weighing 25-30 g which were obtained from Eciyes University, Hakan Çetinsaya Laboratory Animal Care Facility, Kayseri, Turkey. The ambient temperature (22±1°C) and the humidity (50-60%) of the room was maintained with a light–dark cycle of 12:12 h (lights on at 07:00). Animals had free access to standart food and water except for the short time that they were removed from their cages for testing. All testing was conducted between at 09:00 - 12:00 h. Animals were transferred to the testing area in their home cage and allowed to adapt to the new environment for at least 1 h before testing. The forced swimming test (FST) was used to evaluate the antidepressant-like activity (32). The animals were individually forced to swim in an open cylindrical glass container (diameter 10 cm, height 25 cm), containing 10 cm of water at 25±1°C for 6 min and immobility time was recorded during the last 4 min. Each mouse was judged to be immobile when it ceased struggling and remained floating motionless in the water, making only those movements necessary to keep its head above water. The test was performed by the same experimenter, who was unaware of the treatment administered.

The animals were randomly divided into 8 groups having 6 mice in each group consisting equal number of either sex of mice. Mice were injected with saline + vehicle (control), two doses of amitriptyline (7.5 mg/kg and 15 mg/kg), adenosine A_{2A} receptor selective antagonist SCH 58261 2 mg/kg, SCH 58261 2 mg/kg + amitriptyline 7.5 mg/kg, two doses of adenosine (2 mg/kg and 10 mg/kg), adenosine 2 mg/kg + amitriptyline 7.5 mg/kg intraperitoneally (i.p.). In combined groups SCH 58261 (Sigma) was injected 15 minutes before amitriptyline (Sigma) whereas adenosine (Sigma) was co-injected with the amitriptyline. Thirty minutes after last injections mice were taken to the FST test. Amitriptyline HCl and adenosine were dissolved in saline, SCH 58261 was dissolved in dimethyl sulphoxide (DMSO, Sigma) and then diluted in saline (the final concentration of DMSO was 20%). All drugs were injected i.p. in a volume 4 ml/kg. Control group was injected with saline and saline containing 20% DMSO as a vehicle.

STATISTICAL ANALYSIS

Statistical analyses were performed using IBM SPSS software ver. 22.0. Results are expressed as arithmetic mean ± standard error of mean (S.E.M.) of immobility time in seconds for each experimental group. The data normality was assessed using Shapiro-Wilk's test. Homogeneity of variances was evaluated by Levene's test and data were analysed by one-way analysis of variance (ANOVA). Post-hoc comparison was carried out with the Tamhane's T2 test. Significance levels were set at $p < 0.05$.

RESULTS

Amitriptyline decreased immobility time compared to control group at both doses (7.5 and 15 mg/kg) significantly ($p<0.05$), however, there was no significant difference between two doses of amitriptyline ($p>0.05$). SCH 58261 also decreased the immobility time, however, it was not statistically significant ($p>0.05$). Combined treatment of SCH 58261 and 7.5 mg/kg amitriptyline produced stronger inhibition of immobility than 7.5 mg/kg amitriptyline alone ($p <0.05$).

Adenosine at 2 or 10 mg/kg i.p doses decreased the immobility time slightly, however, it was not

statistically significant when compared to control group (p>0.05). Coadministration of 2 mg/kg adenosine with amitriptyline 7.5 mg/kg decreased the antidepressant effect of amitriptyline, however, it was not significant statistically at applied dose. Results are presented in the Table 1.

Table 1. Effects of treatment with saline (SF), amitriptyline 7.5 mg/kg (Amit 7.5), amitriptyline 15 mg/kg (Amit 15), SCH 58261 2 mg/kg + amitriptyline 7.5 mg/kg (SCH+Amit 7.5), adenosine 2 mg/kg + amitriptyline 7.5 mg/kg (Aden 2+Amit 7.5), SCH 58261 2mg/kg (SCH), adenosine 2 mg/kg (Aden 2), and adenosine 10 mg/kg (Aden 10) on immobility time (seconds) in the FST test in mice (n=6). Same letter represents the no significant difference (p>0.05), different letters show significant difference between groups (p<0.05). Homogeneity of variance was tested by Levene's test (p= 0,03). One-way ANOVA with posthoc Tamhane's T2 test was performed to evalute statistically significance between groups.

Groups	**SF**	**Amit 7.5**	**Amit 15**	**SCH+ Amit 7.5**	**Aden 2+ Amit 7.5**	**SCH**	**Aden 2**	**Aden 10**
Mean ± S.E.M	160,8 [c] ± 9,8	80,8 [a] ± 3,7	62,3 [ab] ± 4,9	48,6 [b] ± 2,71	130,8 [c] ± 13,7	143,6 [c] ± 4,4	152 [c] ± 4,5	98,1 [ad] ± 5,6

increase the levels of adenosine in the CNS or activates the adenosine receptors (8,10), so pharmacologically blockade of the A_{2A} receptors with ineffective dose of selective antagonist may increased the antidepressant like effect of amitriptyline. It may be the result of the inhibition of the neuronal uptake of adenosine by amitriptyline which would lead to enhanced stimulation of A_1 receptors in the brain when A_{2A} receptors were blocked with the selective antagonist. Additionally, it has recently been shown that amitriptyline binds to A_{2A} receptors (30), so combining with selective receptor antagonist may have increased antidepressant like efficacy. We used ineffective dose of antagonist as it was shown that acutely administered 3 and 5 mg/kg i.p SCH 58261 exerts antidepressant like effect in FST in CD21 mice (20). In contrast to result of Kaster et al. (26), antiimobility effects of systemically applied adenosine at 2 and 10 mg/kg doses were not significant. Combination of 2 mg/kg adenosine increased the immobility time of the amitriptyline, however, it was not significant statistically. As the studies related with antidepressant -like activity of drugs had been performed with male animals, we performed the groups consisting equal number of male and female mice. Different results of preclinical studies related with adenosine could be ascribed to the diverse dose range, different mice strains and gender used. We didn't perform locomotor activity tests in our study as it was shown that SCH 58261 at 0.312 to 2.5 mg/kg dose ranges failed to produce a significant effects on locomotion in mice (39).

DISCUSSION

Amitriptyline is a widely used TCA drug that has been approved for treatment of MDD but also used in several chronic pain conditions (2,5). Although antidepressant effect of amitriptyline is generally associated with the inhibition of serotonin and norepinephrine uptake in the CNS, the monoaminergic hypothesis is not enough to explain the mechanism of depression and antidepressant drugs' action (33). Amitriptyline has multiple molecular targets; antagonistic effects at histaminergic, muscarinic, α_1-adrenergic, and serotonin receptors at nanomolar concentrations and blocks sodium, calcium, and potassium ion channels. At micromolar concentrations also blocking the uptake of the adenosine (2,3). Evidences suggesting that part of the actions of amitriptine could be mediated by adenosinergic neurotransmission enhancement (10,34-37). Moreover, it was demostrated that chronic imipramine treatment enhanced the presynaptic inhibitory effect of adenosine in the hippocampus of rats (38).

Adenosine exerts its central effects via abundantly found A_1 and A_{2A} receptors in the CNS (11). A_1 receptors are widely located in the brain and mediate inhibitory actions while A_{2A} receptors mediate excitatory actions of adenosine. So, the synaptic transmission and neurotransmitter release are affected by through the activation of oppositely working adenosine receptor subtypes (11-14). Adenosine integrates and controls many of normal brain functions and also plays important roles in pathologic conditions such as ischemia, epilepsy, Parkinson's disease and depression (11,15,16).

In our study we found that antidepressant-like effect of amitriptyline was increased by the pretreatment with the selective A_{2A} receptor antagonist, SCH 58261. In accordance with the studies related with the effects of amitriptyline in pain models, amitriptyline seems to

In general, adenosine and its analogues produced `depressant' effects in animal models. Stimulation of adenosine receptors or increase of adenosine levels induce a state of `learned helplessness' (18) or administration of adenosine and analogue increase the immobility time in the FST in mice (17). Additionally, adenosine A_{2A} receptor antagonists produce an antidepressant-like effect in mice and adenosine A_{2A} receptor knockout animals have less immobility time in FST and TST (19-23) and in reserpine induced depression model (24).

In consistently, behavioral and synaptic alterations of

CUS were prevented by the selective A_{2A} receptors antagonist istradefylline (KW6002), and selective A_{2A} receptor deletion in forebrain neurons (25). It was reported that unpredictable chronic mild stress (UCMS) promoted an up-regulation of striatal A_{2A} receptors and induced depressive-like behavior in rats (40). It was also shown that the platelets of depressive patients have a curtailed response to A2A receptor agonists (41). Istradefylline decreased immobility time in the animal depression models and also significantly improved the inescapable shock induced escape deficit with a degree efficacy to an equivalent dose of desipramine and imipramine (22,23). Moreover, similar to our results istradefylline increased the antidepressant-like effects of MAO-B inhibitor or selective serotonin reuptake inhibitor antidepressants at doses that is ineffective when applied alone (22). Due to the ability of interaction with dopaminergic, and serotonergic systems adenosine has complex effects in depression (42,43). It was hypothesized that the antidepressant-like effect of selective A_{2A} receptor antagonists is linked to an interaction with dopaminergic transmission as A_{2A} receptor and dopaminergic D_2 receptor are co-localized in the dorsal-ventral striatum (44). Antidepressant-like effects of SCH 58261 prevented by the dopamine D_2 receptor antagonist haloperidol in the FST (19) and the action of dopamine agonists was enhanced by the A2A receptor antagonists via the dopaminergic D_2 receptor (44).

It was reported that adenosine at high doses (25-100 mg/kg, i.p) prolonged the immobilization time in mice FST and the effect was blocked by the non-selective adenosine receptor antagonists, caffeine and theophylline. In contrast, systemic lower doses (5–10 mg/kg) or intracerebroventicular (0.01–10 µg) administrations caused immobility time reduction in the depression tests apparently mediated by an interaction with A_1, A_{2A} and 5-HT_{1A} receptors (26). Some other studies have also showed that A_{2A} receptors activation is implicated in the antidepressant-like effect of adenosine and inosine (27,28). This contraversial results might be due to animal model and/or procedures employed and the doses drugs.

Inhibition of adenosine uptake in brain cortical synaptosomes (7), explains the depressive effects of TCA drugs on the firing rate of cortical neurons in vivo, and enhance the inhibitory actions of adenosine (8). By the means of microdialysis it was shown that amitriptyline inhibits uptake of adenosine and increases extracellular levels of adenosine (7,10). Studies have shown that endogenous adenosinergic system involves in the effects of amitriptyline in inflammatory and neuropathic pain models (10,34). It was shown that local administration of amitriptyline inhibits adenosine uptake and enhanced the extracellular tissue levels of adenosine in the rat hinpaw (10). It was also demostrated that both peripheral and systemic administration of amitriptyline induced antiallodynic effect in rat model of painful diabetic neuropathy and partially reversal of effects by the caffeine suggest the involvement of endogenous adenosine in the action of amitriptyline (35). Additionally, studies showed the role of endogenous adenosine in amitripyline induced cardiovascular toxicity as hypotension and QRS prolongation by amitriptyline-poisoned rats were reversed by selective adenosine A_1 and A_{2A} receptor antagonists, (36) and plasma levels of adenosine increased in poisoned rats (37). On the other hand, it was shown that some TCA drugs can bind to adenosine receptors (29,30) and also amitriptyline inhibits ATP and ADP hydrolysis at 0.1–1.0 mM concentrations in cerebral cortex of adult rats in in vitro conditions (31).

Our results indicate that pretreatment with the ineffective dose of adenosine A_{2A} receptor antagonist may induce a more pronounced antidepressant-like activity of amitriptyline. It may be the result of the inhibition of neuronal adenosine uptake or activation of adenosine receptors by amitriptyline. Our finding may be of particular importance in the case of drug resistant patients. Additionally, combination may offer significant antidepressant effect without increasing the dose of amitriptyline so may lead to reduced systemic side effects of the drug. However, we need further studies to show interaction between antidepressants and adenosine on depression clinically by the mechanism.

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Declaration of interest

Author confirms that there is no conflict of interest.

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