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Effect of Scopolamine and Mecamylamine on Antidepressant Effect of Rivastigmine in a Behavioral Despair Test in Mice

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Rivastigmine can decrease the severity of depression in Alzheimer's disease patients. Apart from the monoamines hypothesis of despair, it is well known that the cholinergic system is also responsible. Thus regarding the antidepressant effect of rivastigmine, the aim was evaluating the influence of scopolamine (muscarinic cholinergic receptors antagonist) and/or mecamylamine (nicotinic cholinergic receptors antagonist) co-administration with rivastigmine on depression outcome. Depression was evaluated by the tail suspension test (TST) in male mice. Immobility time was recorded manually which represents the time that animal stops attempts for escape from the unpleasant situation. Imipramine as the reference antidepressant decreased this time by increasing the hope for escape in the animal. Rivastigmine (0.75 mg/kg,sc), scopolamine and/or mecamylamine (0.5 and 1 mg/kg, ip, respectively) were injected before the TST. Rivastigmine considerably reduced the immobility time compared with the control group which indicated its antidepressant effects. The combination treatment of rivastigmine and scopolamine also reduced the immobility time compared with control. Mecamylamine co-administration with rivastigmine increased the immobility time which indicated that it prevented the antidepressant effects of rivastigmine. Ultimately by using the drugs all together the immobility time notably decreased. Therefore our results proved that nicotine cholinergic receptorstimulation is prominent in the antidepressant effects of rivastigmine. Thus nicotine receptor direct or indirect stimulants could be considered for further researches regarding antidepressants.

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Introduction

Rivastigmine is a, reversible non-competitive inhibitor of acetylcholinesterase (AChE) with carbamate-based structure used to treat mild to moderate dementia in Alzheimer's disease (AD) patients [1]. There are reports indicating that AD patients administered rivastigmine which were evaluated by the Hamilton Depression Scale show reversal in depression [2]. In 2013 a 6-month observational study on 50 patients with mild AD reported that treatment with a rivastigmine patch decreased the frequency and severity of major depressive episodes [3].

Evidently, a complex set of neurotransmitters contributes in the mechanism of depression. Additionally, various drugs that influence despair symptoms act at least in part by changing neurotransmitters and such alterations may be drug related. For half a century, the majority of researches have explained depression with the monoamine hypothesis, which suggests that low levels of brain monoamines, such as serotonin, noradrenaline and dopamine, are responsible for development of symptoms. Currently used antidepressant drugs, such as, tricyclics (TCA), selective serotonin reuptake inhibitors (SSRI), serotonin and noradrenaline reuptake inhibitors (SNRI) promote the monoamine levels [4]. Although their effects on the monoaminergic system is acute, but the mood improving effect of these medications take several weeks to become noticeable [5]. Therefore, the monoamine hypothesis has not been successful to thoroughly explain the nature of despair [6]. Evidence strongly indicates that augmented ACh signaling in humans causes an increased in depressive symptoms [7]. This has been observed following administrating the AChE blocker physostigmine, to patients with depression history, Tourette's syndrome and normal candidates; apparently through increased central acetylcholine levels [7, 8]. Treatment with choline was also occasionally associated with depressive symptoms [9]. Scopolamine is a centrally acting inhibitor of the muscarinic cholinergic receptor. A review article by Jaffe and colleagues 2013 concluded the rapid antidepressant effects of scopolamine in depression [10]. Therefore on the basis of previous studies the cholinergic system and its receptors must be considered carefully regarding depression.

Although it has been determined that rivastigmine effect on the hippocampal serotonergic system is important in its antidepressant effects [11]. Understanding the possible role of cholinergic receptors on rivastigmine antidepressant effect is also crucial. Hence, the following research aimed to understand wheatear antidepressant effect of rivastigmine, is in connection to muscarinic (MR) or nicotinic (NR) cholinergic receptors.

Materials and methods

Animals

Male white mice weighing 25-30 g were housed in cages of six at 21± 2 ºC in a 12 h light-dark cycle with the lights on at day time 6 am-6 pm. Tap water and standard food pellets were available ad libitum. Tests were performed only after the mice had acclimated to the above environment for at least 2 days. In order to minimize circadian rhythm influence, all experiments were conducted between 08:00 and 13:00 h, in the pharmacology laboratory. Minimum of six mice were used for each treatment group. All animal procedures were performed in accordance with guidelines for the Care and Use of Laboratory Animals Issued by Isfahan University of Medical Sciences.

Tail suspension test (TST)

The TST is a model of despair where animals cannot escape from an unpleasant condition. A reduction in struggling behavior is deduced as a reduction in intrinsic motivation to escape the situation. Briefly modified, mice were suspended 50cm above the floor by a clip attached, approximately 1 cm from the tip of the tail [12]. The duration that mice remained immobile was recorded during the last 4min of the total 6 min test. The latency to the first immobility episode and the duration of immobility over a 6 min period were continually manually measured. An animal was rated as immobile when there was no movement of the head, extremities or the trunk. Few numbers of animals showed tail climbing

behavior, but when this was observed, the data were omitted.

Locomotion test

The motor activity of mice was assessed in a rectangular, illuminated, plastic enclosure divided into 15 zones in a 5×3 grid formation [13]. Each zone was 9cm×10 cm. Mice were placed facing towards the wall in the closest corner to the experimenter and were allowed to explore the field for 3 min. The number of zone entries and rears on hind-legs were recorded manually. The total activity was calculated by summing the zone entries (horizontal exploration) and rears (vertical exploration).

Drug therapy

Rivastigmine tartrate (Sigma-Aldrich, Germany) 0.75 mg/kg was injected sc 30 min before the TST. Scopolamine (20 mg/ml, Tehran-shimi, Iran) 0.5 mg/kg, and mecamylamine hydrochloride (Sigma-Aldrich, Germany) 1 mg/kg were injected ip 10 min after rivastigmine administration (20 min before the TST). Imipramine hydrochloride (Sigma-Aldrich, Germany) 10 mg/kg was injected ip 30 min before TST. The solvent for all preparations were normal saline. All animals were injected 10 ml/kg according to their weights.

There were 8 study groups which comprised min of six animals as following: 1) the control group, 2) the imipramine group, 3) the rivastigmine group, 4) the scopolamine group, 5) the mecamylamine group, 6) the group administered rivastigmine, andscopolamine,7) the group administered rivastigmine, and mecamylamine,8) the group administered rivastigmine, scopolamine , and mecamylamine.

Data processing and statistical analysis

Results were expressed as group mean ± SEM. All results were analyzed by a one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison tests, P values less than 0.05 were considered significant. The software' used for data analyzing and making graphs was Graphpad Prism 6.

Results

In order to evaluate the drug effect on depression in the TST the sum of immobility time was measured for each drug alone (fig. 1A). Imipramine as the positive control drug obviously reduced the immobility time (51s ± 10; $p < 0.001$ vs control group), thus the TST protocol accuracy was confirmed. Rivastigmine and scopolamine significantly reduced the immobility time compared to control (N/S group), 90s ± 5.6 p <0.01 and 96s ± 9.5 p <0.05 respectively. Mecamylamine did not reduce the immobility time in the TST (128s ± 3.3).

The latency to immobility was increased by all of the drugs as it can be seen in figure 1B. Interestingly although mecamylamine did not improve immobility but it increased the latency in the TST to 123s ± 4.8 which differed significantly with normal saline group 33s ± 4.8 ($p < 0.001$).

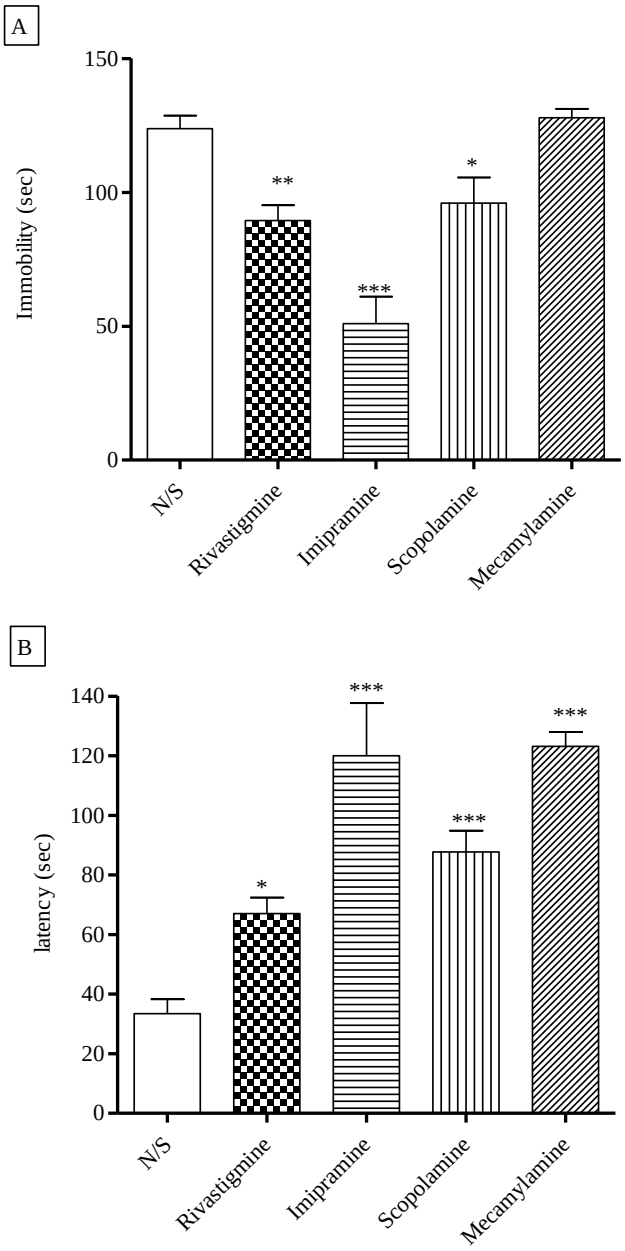

Fig. 1. The effect of drugs alone on depression behavior in the tail suspension test. A: The total time animals were immobile during the last 4 min of the total 6 min test was recorded. B: Latency; time it takes until the animal becomes immobile. Number of animals in each group was 6. Control animals received normal saline (N/S). Results are expressed as group mean ± SEM and analyzed by ANOVA followed by Tukey's comparison tests. (*) $p < 0.05$, (**)$p < 0.01$, and (***) $p < 0.001$ represents difference compared with control group.

Figure 2 A shows the immobility time in TST when scopolamine and/or mecamylamine were added to rivastigmine. Combination of rivastigmine and scopolamine clearly decreased the immobility time (84s ± 0.5) compared with control (126s ± 1.9; $p < 0.001$). Mecamylamine reversed the beneficial effects of rivastigmine on depression as their combination increased the immobility time (131s ± 1.1). Ultimately using the drugs all together the immobility time decreased to 63s ± 11 which was significantly lower than control ($p < 0.001$). As presented in figure 2B this combination

also significantly increased the latency to immobility in the TST (67s ± 4.2 vs control group, 39s ± 4.6; $p < 0.001$).

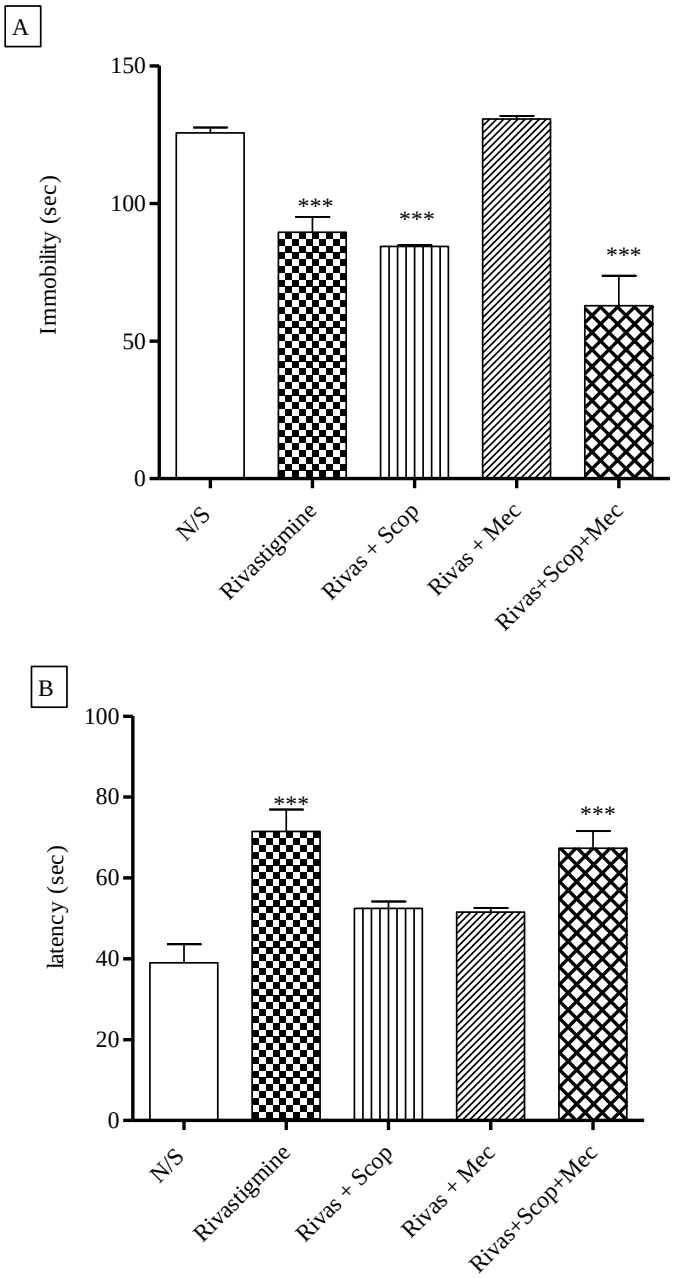

Fig. 2. The effect of drugs co-administration with rivastigmine on depression behavior in the tail suspension test. A: The total time animals were immobile during the last 4 min of the total 6 min test was recorded. B: Latency; time it takes until the animal becomes immobile. Number of animals in each group was 6. Control animals received normal saline (N/S). Results are expressed as group mean ± SEM and analyzed by ANOVA followed by Tukey's comparison tests. (***) $p < 0.001$ represents difference compared with control group. Abbreviations: rivastigmine = rivas, scopolamine = scop, mecamylamine = mec.

By using each drug individually total activity count, the sum of zone entries and hind-leg rears, was not significantly different from controls (93 ± 4.8) (table 1). Combination of the drugs made significant change on the total locomotion count (table 1). Combining scopolamine and/or mecamylamine with rivastigmine although significantly reduced locomotion count 15 ± 1.5, 24 ± 1.2 and 50 ± 6.4 respectively but these values were also significantly higher than zero in the simple t- test ($p < 0.001$). This would indicate that the combination of the drugs has caused partial sedation in the animals.

Discussion

In the tail suspension model of despair used in these experiments rivastigmine by lowering the immobility time proved to have antidepressant property. Scopolamine alone also showed antidepressant effects and the combination treatment of rivastigmine and scopolamine also reduced the immobility time in TST. Mecamylamine by itself did not have benefits on despair, and its co-administration with rivastigmine prevented its antidepressant effects. Therefore NR is involved in rivastigmine antidepressant effects. Using the three drugs together again showed beneficial effects in the animal model of despair.

Imipramine as a TCA significantly reduced the immobility time and caused an increase in the latency to immobility, this part of the experiment confirmed the accuracy of our mice despair model. Rivastigmine decreased the immobility time in the TST, which indicates that it has antidepressant effect (fig. 1). Previous results support this; an observational study reported that rivastigmine patch alone decreased the intensity of major depressive episodes in 50 patients with mild AD [3]. Animal studies also proved antidepressant effects of rivastigmine in forced swimming test [14]. They determined that, rivastigmine not only enhances the cholinergic system but also repairs serotonergic system of the hippocampal, which improves depressive behaviors, in AD patients. Rivastigmin as an AchE inhibitor cause an increase in the Ach level. Previous studies had found links between increase brain Ach and depression [8, 9]. The reason behind rivastigmine antidepressant effect could be related to its indirect effect on cholinergic receptors or direct effects on other receptors.

Scopolamine as a competitive muscarinic receptor antagonist acting centrally also showed beneficial effects on despair (fig. 1). This is in connection to a previous systematic review indicating that scopolamine is an efficient antidepressant and shows its effects on depression as fast as 3 days [10]. This part of study was performed as completion to our main goal and in order to confirm scopolamine antidepressant effect by the TST. Using scopolamine together with rivastigmine still showed beneficial effects on despair (fig. 2). By inhibiting the muscarinic receptors with scopolamine the increased Ach level caused by rivastigmine, will have it main effects on the NRs. Therefore at least some part of beneficial effects of rivastigmine could be because of the NRs stimulation. In order to support this view the next part of the experiments took place by using mecamylamine, a NR antagonist.

Mecamylamine on its own did not have beneficial effects on despair in the TST (fig. 1). While it prevented the beneficial effects of rivastigmine on depression, when they were co-administrated (fig. 2). This was an interesting approach, although previous studies linked rivastigmine mood effects to serotonin and its 5-HT1A receptor-dependent manner [11, 14], our study proved the role for NR to be also vital.

On the other hand, findings about NR on depression are truly conflicting. The story of the link between smoking and depression is not new. Nicotine in tobacco binds to NRs and activates and/or desensitizes it. Previous clinical studies have revealed that symptoms of depression are reduced by nicotine patch, even in depressed non-smokers [15]. Administrating low levels of nicotine chronically (like the nicotine delivering system in patch) seems to desensitize NRs rather than activating it [16, 17]. Other researches provided additional evidence for antidepressant-like effects of nicotine and they came up with a link between striatal NRs and high alcohol intake [18]. Our results were also in favor of the beneficial effects of NR activation in order to improve depressive behavior. Using the three drugs together

(rivastigmine, scopolamine and mecamylamine) again decreased the immobility time in TST that indicated antidepressant effects (fig. 2). It could be deduced from the final results that although increased level of Ach could not stimulate the NRs (antagonized by mecamylamine) but antagonizing muscarinic receptors by scopolamine was essential in order to induce antidepressant effects. Our final experiment also corroborated the previous findings indicating the effects of rivastigmine on the serotonin receptor [11, 14]. In other words although the NR and MR were blocked rivastigmine antidepressant effects persisted this could be because its effects on other noncholinergic receptors and also because of the effects of scopolamine itself.

Locomotor test was performed in parallel with TST to verify the immobility of animals caused by despair from immobility caused by sedation (table 1). According to our experiment design the drugs alone did not affect the locomotion, and groups with high immobility in the TST test had normal movements. Low dose scopolamine in other studies also proved to increase locomotion in the open field [19]. But drug combination caused a reduction in animals' locomotor activity. By the increase in the ACh level caused by rivastigmine, scopolamine and mecamylamine showed decrease in the locomotor activity. Mecamylamine combination with nicotine prevented nicotine induced sensitization in the locomotor test [20]. Thus their central sedative effects were pronounced when they were combined.

Table 1. The open field locomotor activity.

	Hind-leg rears	Zone entry	Total activity
N/S	25 ± 1.2	68.3 ± 4	93 ± 4.8
Imipramine	33 ± 2.7	92.8 ± 5.1*	113 ± 14
Rivastigmine	20 ± 2.3	50.8 ± 3.8*	79 ± 5
Scopolamine	25 ± 2.8	68.2 ± 5.7	93 ± 7.5
Mecamylamine	32 ± 0.6	83.3 ± 2.1	115 ± 2.4
Rivas + Scop	1.8 ± 0.2**	13 ± 1.4**	15 ± 1.5***
Rivas + Mec	6.4 ± 0.5**	18 ± 0.8**	24 ± 1.2***
Rivas + Scop + Mec	9 ± 1.8**	41 ± 5.1*	50 ± 6.4***

The zone entries and hind-leg rears were count in mice for 3 min, the total activity is the sum of zone entries and rears. Number of animals in each group was 6. Control animals received normal saline (N/S). Results are expressed as group mean ± SEM and analyzed by ANOVA followed by Tukey's comparison tests. (*) $p < 0.05$, (**)$p < 0.01$, and (***) $p < 0.001$ represents difference compared with control group. Abbreviations: rivastigmine = Rivas, scopolamine = Scop, mecamylamine = Mec.

Conclusion

In conclusion stimulation of the NRs indirectly by rivastigmine at least in part in our experiments had antidepressant effects. Although on the basis of our experiments following distinct therapies molecular studies were not performed and Ach was not measured in different brain regions which are highly suggested. Thus indirectly NR stimulants must be considered in further researches for antidepressants. This study suggests that NRs could be promising targets for the development of novel approaches for treatment of depression.

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Conflict of interest

Authors certify that there is no conflict of interest in relation to this article.

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