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EFFECTS OF THE AGENTS INFLUENCING THE SEROTONERGIC AND CANNABINOID SYSTEMS ON MEMORY IN THE AVOIDANCE TEST IN MICE

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Adult male albino mice in a shuttle box system were used for examination of learning for avoidance behavior and its deactivation. We measured the step-through latency in the acquisition of the task (STLa) before injections of the drugs tested (fluoxetine and URB597 (a serotonin reuptake inhibitor, SSRI, and an agent preventing decomposition of endocannabinoids, respectively) and the respective latency 24 h later after injections of these agents (STLr); total time spent in the dark compartment (TDC) was also measured in these situations. In mice that received fluoxetine (5, 10, and 20 mg/kg), the STLr were longer than those in the control, and the difference was significant at 10 mg/kg. Injections of URB597 decreased the STLr and, at medium and high doses (0.3 and 1.0 mg/kg), provided significant differences. All doses of fluoxetine led to significant decreases in the TDC values, while injections of URB597 increased this index (at 0.3 and 1.0 mg/kg, the shifts were significant). Combined injections of fluoxetine and URB597 (5 + 0.1, 10 + 0.3, and 20 + 1.0 mg/kg) increased the STLr values and decreased TDC values to the levels comparable with those at isolated injections of fluoxetine in the respective doses. Thus, fluoxetine improved memory, while URB597 impaired it; fluoxetine is capable of nullifying negative effects of URB597.

Keywords: serotonin, endocannabinoids, inhibitory avoidance test, acquisition, retention, memory.

INTRODUCTION

Serotonin is one of the most important neurotransmitters involved in the memory and learning processes [1]. Cannabinoids also play important roles in the control of neurobehavioral phenomena [2]. The relationship between cannabinoids and the memory system was examined in a few studies. It was reported that endocannabinoids (like anandamide) impair learned behavior [3]. At the same time, another study showed improvement in cognition and memory under the influence of these agents [2]. The serotonin system affecting the memory structures plays an important role in mood disorders and dysfunction of serotonergic neurotransmission in various mental diseases [4]. This is why serotonin reuptake inhibitors (SSRIs) are most frequently used in the treatment of major depression [5]. We used fluoxetine as one of the SSRIs in the current study. This agent demonstrated no binding affinity in the brain for any other major receptor classes, and it is characterized by a relatively long half-life in the rat [6]. There are, however, some reports that serotonin (5-HT) providing activation of 5-HT receptors impaired short-term memory, and blocking of the respective effects may intensify the antidepressant effect of SSRIs and improve cognition [7]. It was reported that fluoxetine improved cognition and spatial memory [5], but some results are contradictory [8, 9].

Type 1-cannabinoid receptors (CB1) and 5-HT receptors are distributed in the hippocampus, and both of them exert effects on the memory and learning functions. Thus, it was suggested that their combined activation may affect learning and memory in a complex mode [10]. This aspect has not been studied well until now. So, we decided to study the effects of combined potentiation of the effects of endogenous serotonergic and cannabinoid systems on memory in mice.

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METHODS

Animals. Male albino mice (body mass 20-30 g) were used in this study. The animals were housed five per cage and maintained at 20 ± 2°C and at a 12/12-h light/dark photocycle (lights on 07:00 a.m.). Water and food were available *ad libitum*. All mice were acclimatized to the environment for at least 10 days prior to the start of behavioral testing. They were trained to perform the step-through inhibitory avoidance task (IAT). The mice received single intraperitoneal (i.p) injections of saline, fluoxetine (5.0, 10, or 20 mg/kg), URB597 (0.1, 0.3, or 1.0 mg/kg), or of their combination (fluoxetine + URB597, 5 + 0.1, 10 + 0.3, or 20 + 1.0 mg/kg). After this, their retention of the memory performance was evaluated.

Inhibitory Avoidance Apparatus. The apparatus and procedure were basically the same as those in our previous studies [11-13]. The apparatus consisted of a lighted chamber and a dark one. Between two chambers, there was a rectangular opening that could be closed by an opaque guillotine door. The floor of both chambers was equipped with stainless steel rods, and the floor of the dark chamber could be electrified.

Mice were placed in a lighted compartment of the apparatus facing away from the door; 5 sec later, the guillotine door was raised. Once the mouse entered the dark compartment, the door was closed, and the mouse was taken from the dark compartment into its home cage. The habituation trial was repeated 30 min later and followed (after the same interval) by the first acquisition trial. The entry latency to the dark compartment (step-through latency, STL) was recorded when the animal had placed all four paws on the floor of the dark compartment. After an animal spontaneously entered the dark compartment, the guillotine door was lowered, and a mild electrical shock (0.6 mA) was applied for 3 sec. The mouse was retained in the apparatus and received a foot-shock each time the animal re-entered the dark compartment. Training was terminated when the mice remained in the light compartment for consecutive 120 sec.

Experimental Procedures. The animals were divided into 10 groups (n = 8 in each). They were trained for the step-through IAT. The STL of the first acquisition trial and the number of trials to IAT acquisition were recorded.

The retention test was performed 24 h after the IAT acquisition trial. The animals received single i.p injections of the above-mentioned agents 30 min before the retention test. Then, each mouse was placed in a lighted chamber as in the IAT training; 5 sec later, the guillotine door was raised. Then, the STL and time spent in the dark compartment (TDC) were recorded up to 300 sec. If the mouse did not enter the dark compartment within this time interval, the retention test was terminated, and a ceiling score of 300 sec was assigned.

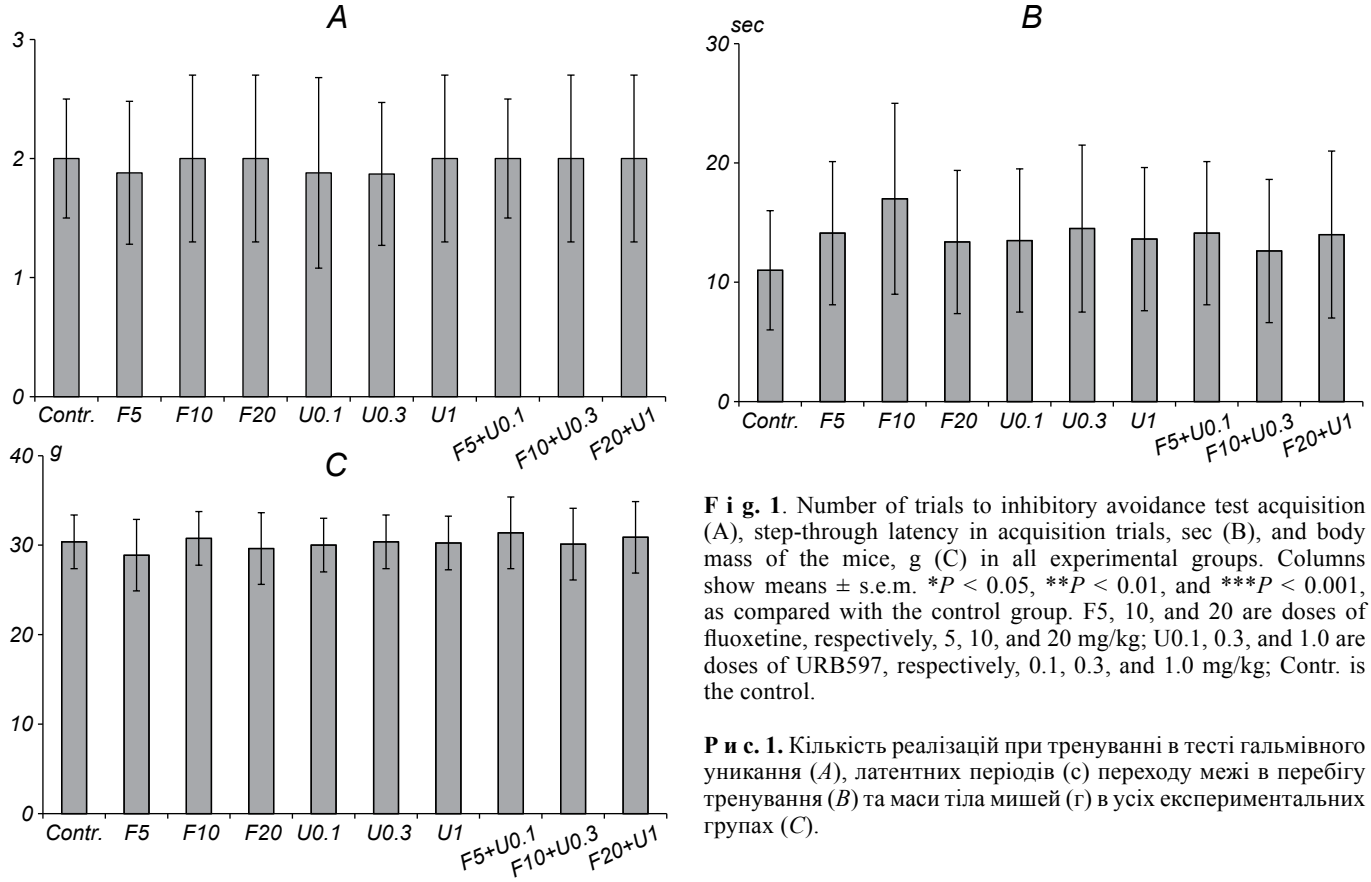
Statistical Analysis. Statistical significance of the differences of each measured parameter between experimental groups was estimated by one-way ANOVA or Kruskal–Wallis non-parametric ANOVA and followed by the Tukey or Dunn tests for multigroup comparison when appropriate. The zero hypothesis probabilities below 0.05 were considered significant. All data presented in the figures are given as means ± s.e.m.

RESULTS

Acquisition. There were neither significant difference in the number of trials to acquisition nor in the STL in the acquisition of the task (STLa) between the experimental groups. There was also no difference in the mean body mass among all groups ($P > 0.05$; Fig. 1).

Retention. In the retention test done 24 h after the training period, the one-way ANOVA test indicated that there was a significant difference in the STL (STLr) values between the experimental groups (Fig. 2). The Tukey *post-hoc* test revealed that the STLr in the fluoxetine (10 mg/kg)-treated group was significantly longer than that in the control ($P < 0.05$). The values of STLr in the URB597 (0.3 and 1.0 mg/kg)-injected groups were significantly shorter in comparison with the control ($P < 0.05$ and $P < 0.01$, respectively). In the three (fluoxetine + URB597)-treated groups, the STLr values were significantly longer than those in the control ($P < 0.05$).

Statistical comparison of the TDC by one-way ANOVA indicated that there was a significant difference between experimental groups (Fig. 2). The Tukey *post-hoc* test showed that, in the fluoxetine (5, 10, and 20 mg/kg)-treated groups, the TDCs were highly significantly shorter than those in the control ($P < 0.01$, $P < 0.001$, and $P < 0.01$, respectively). On the other hand, in the URB597 (0.3 and 1.0 mg/kg) mice, the TDCs were much longer than those in the control ($P < 0.001$). At the same time, injections of fluoxetine+URB597 in all the three combinations used led to highly significant ($P < 0.001$)



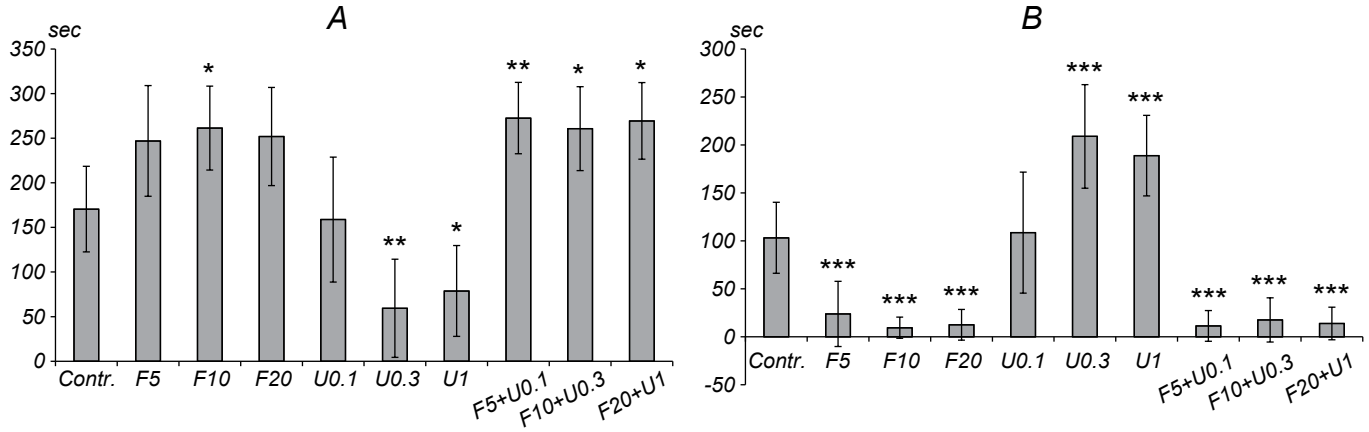
F i g. 1. Number of trials to inhibitory avoidance test acquisition (A), step-through latency in acquisition trials, sec (B), and body mass of the mice, g (C) in all experimental groups. Columns show means ± s.e.m. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$, as compared with the control group. F5, 10, and 20 are doses of fluoxetine, respectively, 5, 10, and 20 mg/kg; U0.1, 0.3, and 1.0 are doses of URB597, respectively, 0.1, 0.3, and 1.0 mg/kg; Contr. is the control.

Р и с. 1. Кількість реалізацій при тренуванні в тесті гальмівного уникання (*A*), латентних періодів (с) переходу межі в перебігу тренування (*B*) та маси тіла мишей (г) в усіх експериментальних групах (*C*).

shortenings of TDCs to the levels close to those in the "pure" fluoxetine-injected groups.

DISCUSSION

Different neurotransmitters are involved in the memory and learning processes, and one of the important ones, from this aspect, is serotonin [1]. On the other hand, cannabinoids also play important roles in the neurobehavioral processes [2]. The cannabinergic system components, such as its ligands and receptors (CB1s), are distributed in the hippocampus and other structures related to memory; it is well known that



F i g. 2. Values of the step-through latency in the retention test, sec (A) and total time spent in the dark compartment, sec (B) in all experimental groups. Other designations are the same as in Fig. 1.

Р и с. 2. Значення латентних періодів (с) переходу межі у відставленому тесті (*A*) та загального часу (с), проведеного в темному відсіку (*B*) в усіх експериментальних групах.

the hippocampus is an important center for learning and memory formation. Type 1-cannabinoid receptors are present in presynaptic terminals of neurons of the hippocampus and striatal complex [14].

Our study showed that the STLr values (STL of going to the dark compartment after injections of URB597 and fluoxetine) 24 h after the task (IAT) acquisition in groups that received moderate (0.3 mg/kg) and high doses (1.0 mg/kg) of URB597 were significantly shorter than in the control ($P < 0.05$). The TDC values altogether (at low, 0.1 mg/kg, moderate, 0.3 mg/kg, and high 1.0 mg/kg doses of URB597) were greater than in the control group ($P < 0.001$), suggesting that endocannabinoids significantly impair memory processing. There are different and controversial communications with respect to the effects of cannabinoids on memory and learning processing. At the same time, it was reported that exogenous cannabinoids disrupt encoding in the process of memorization by altering the functions of a specific type of hippocampal neurons [15], and that endocannabinoids exert a negative effect on the hippocampus-related encoding for short-term memory [15]. In our study, a negative effect of cannabinoids on memory is consistent with earlier reports. Administration of a cannabinoid antagonist SR (SR14176A) attenuated the memory impairment caused by anandamide and improved memory and learning [16]. Local administration of SR (SR14176A) in the delayed radial maze task caused the blockade of CB1 receptors and enhanced consolidation of spatial memory [17]. Another study reported that endocannabinoids impaired memory and caused extinction of previously trained behavior [3]. Studies on CB1-knockout mice in the objective recognition task showed that these animals demonstrated better memory than the wild-type control; spatial memory was facilitated [18]. In other studies, it was described that administration of a CB1 antagonist (AM251) provided the blockade of extinction of memory, improvement of the performance related to short-term memory, facilitation of memory, and reversion of the cognition deficits caused by cannabinoid agonists [16, 19, 20]. All the above reports agree with our results. Nonetheless, it should be mentioned that we found some reports that are in contrast. As was reported earlier, administration of a cannabinoid antagonist impaired the spatial learning function [21], and a CB1 receptor antagonist negatively influenced memory in certain tests [22].

The serotonergic system, by acting via the prefrontal cortex, dorsal hippocampus, and amygdalar complex [23], plays important roles in mood disorders. Dysfunction of serotonergic neurotransmission induces various mental disorders [4]; thus, SSRIs became the most frequently used agents for treatment of major depression [5]. The SSRIs increased the amount of 5-HT receptors. The effect of 5-HT can be explained by its high level in brain structures involved in cognition (hippocampus and temporal cortex) [24, 25]. The 5-HT receptor overactivation impaired short-term memory, and blocking of these receptors may improve the antidepressive effect of SSRI and enhance cognition [7].

Our results showed that the STLr value after injections of URB597 and fluoxetine into the animal group that received a moderate dose of fluoxetine (10 mg/kg) was longer than that in the control group; the analogous trend was observed in other fluoxetine-injected groups. In all three groups that received low (5 mg/kg), moderate (10 mg/kg), and high (20 mg/kg) doses of fluoxetine, the TDCs were shorter than in the control group suggesting an improvement effect on the memory function by fluoxetine. Most studies reported comparable results. Fluoxetine improved cognition and spatial memory, and SSRIs partly removed memory deficits in patients with various pathological conditions [5, 26]. At the same time, some reports are in contrast with our findings [27]. Fluoxetine was reported to impair different types of memory and cognition in patients with various mental disorders [8, 28, 29]; so, there are some contradictions also in this field. Therefore, the mechanisms of the actions of the serotonergic system and cannabinoids and their effects on memory processing and learning remain incompletely identified. Fluoxetine increased neurogenesis in the hippocampus and other regions associated with cognition and memory [30].

We found clear indications that the serotonergic system and endocannabinoid system may provide combined effects. Endocannabinoids affect serotonergic neurons [10]. On the other hand, CB1 and 5-HT receptors are present in the hippocampus, and it seems that their combined activation can affect memory and learning in a complex mode [31]. Fluoxetine increases the amount of CB1s in the hippocampus and, thus, can modify the cannabinoid system [32].

This aspect (combined action of fluoxetine and cannabinoids on memory) was not investigated until

now. This in why we studied the effects of both these factors in the groups that received fluoxetine and URB597 together. Our results showed that the STLr values in all "mixed" groups (5 mg/kg fluoxetine + + 0.1 mg/kg URB597, 10 mg/kg fluoxetine + + 0.3 mg/kg URB597, and 20 mg/kg fluoxetine + + 1.0 mg/kg URB597) were longer ($P < 0.05$) than in the control group. At the same time, the TDCs in all "mixed" groups were shorter than those in the control ($P < 0.001$), suggesting that fluoxetine abolished negative effects of endocannabinoids on memory.

How serotonin affects the cannabinoid system? We suggest that serotonin can do this via its interaction with the dopaminergic and glutaminergic systems [1, 33-35]. On the other hand, fluoxetine can increase the number of CB1 receptors in the hippocampus, and the serotonergic system can modify the cannabinoid system [32]; thus, the serotonergic and cannabinoid systems may affect each other via this mechanism. Other reports may help us to understand the mechanism of such combined activation in memory processing; endocannabinoids have a nerve-protective effect and promote neuronal proliferation. Both the above systems affect neuronal differentiation in the hippocampus and other structures related to memory [7, 23, 36, 37].

Our study showed how the serotonergic system can improve memory; on the other hand, cannabinoids can impair memory. It can be concluded that the serotonergic system nullifies the negative effects of cannabinoids on memory.

Our study has some limitations. For example, we did not study the effects of the above systems on neurogenesis and on other related cerebral phenomena. We suggest, however, that some obtained information may help one to identify in more detail the mechanisms of interaction between the serotonergic system and cannabinoids in future studies.

Based on our own research, we believe that there is a need for further study to determine the combined potential effect of endogenous serotonergic and cannabinoid systems on memory.

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All experimental procedures corresponded to internationally accepted ethical principles for scientific experiments on vertebrate animals.

The authors, N. Rezapoor, S. Shahidi, and A. Komaki, confirm that they have no conflict of interests.

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ВПЛИВИ АГЕНТІВ, ДІЮЧИХ НА СЕРОТОНІНЕРГІЧНУ І КАНАБІНОЇДНУ СИСТЕМИ, НА ФОРМУВАННЯ ПАМ'ЯТІ В ТЕСТІ УНИКАННЯ У МИШЕЙ

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Р е з ю м е

У дорослих білих мишей-самців досліджували навчання поведінці уникання та деактивацію цього процесу в системі із човниковою камерою. Виміряли латентні періоди перетину межі при навчанні дó ін'єкції тестованих агентів – флуоксетину (інгібітора зворотного захоплення серотоніну, SSRS) та URB597 (речовини, що перешкоджає декомпозиції ендоканабіноїдів) і після таких ін'єкцій (STLa і STLr) відповідно; визначали також загальний час, проведений у темному компартменті в даних ситуаціях (TDC). У мишей, які отримували флуоксетин (5, 10 або 20 мг/кг), STLr ставали більшими, ніж у контролі, причому в разі використання 10 мг/кг різниця середніх була вірогідною. Ін'єкції URB597 зменшували значення TDC, і при середніх і високих дозах (0.3 і 1.0 мг/кг) відмінності перевищували рівень вірогідності. Флуоксетин у всіх дозах зумовлював істотне зменшення значень TDC, а ін'єкції URB597 збільшували цей показник (при 0.3 та 1.0 мг/кг зрушення були вірогідними). Комбіновані ін'єкції флуоксетину та URB597 (5 + 0.1, 10 + 0.3 і 20 + + 1.0 мг/кг) призводили до збільшення значень STLr і зменшення TDC до рівнів, порівнянних із тими, які спостерігалися в умовах ізольованих уведень флуоксетину у відповідних дозах. Таким чином, флуоксетин покращував пам'ять, тоді як URB597 порушував її; флуоксетин має здатність нейтралізувати негативні ефекти URB597.

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