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THE EFFECTS OF PIRACETAM ON MORPHINE-INDUCED AMNESIA AND ANALGESIA: THE POSSIBLE CONTRIBUTION OF CENTRAL OPIATERGIC MECHANISMS ON THE ANTIAMNESTIC EFFECT OF PIRACETAM

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ABSTRACT

Aksu F, Gültekin I, Inan SY, Baysal F. The effects of piracetam on morphine-induced amnesia and analgesia: the possible contribution of central opiatergic mechanisms on the antiamnestic effect of piracetam. Inflammopharmacology. 1998;6:53 65.

The involvement of opiatergic mechanisms on the antiamnestic effects of piracetam was investigated in mice. First, the effects of piracetam and naloxone on the amnesia induced by scopolamine, electroconvulsive shock and morphine were evaluated by using elevated plus maze apparatus. Second, the effects of electroconvulsive shock and piracetam on the antinociceptive action of morphine were tested by means of radiant heat tail-flick experiment. Piracetam and naloxone reversed the drug- or electrically-induced amnestic effects. On the other hand, electroconvulsive shock treatment enhanced the antinociceptive effect of morphine while piracetam decreased the same activity. These results suggest an important role of the opiatergic system on the learning and memory process as well as on the antiamnestic effect of piracetam.

Keywords: electroconvulsive shock, morphine, naloxone, piracetam

INTRODUCTION

A nootropic drug, piracetam, which is known to have cognition-enhancing properties, is able to reverse the amnesia induced by electroconvulsive shock (ECS), scopolamine and hypoxia [1]. The mode of action of piracetam and related compounds, however, has not been clearly elucidated. They facilitate cholinergic transmission, a mechanism by which the enhancement of cognition may be produced [2,3]. The cholinergic system may play an important role in memory processes since cholinergic receptor antagonists, atropine and scopolamine, impair memory functions [4] and an acetylcholinesterase inhibitor, physostigmine, facilitates the same parameters [5]. Other transmitters, such as glutamergic, GABAergic, serotonergic, monoaminergic and opiatergic transmitors, may also participate in the cognition-enhancing action of nootropics. The indication for such possible mechanisms arises from the fact that ECS-induced amnesia, in which piracetam has memory-improving effect, can produce many biochemical changes in the central nervous system [6]. Among them, the release of opioid peptides after ECS treatment [7,8] seems to be interesting.

The present study was designed to assess whether the opiatergic system is involved in the effects exerted by piracetam on memory in mice. We employed morphine as an opioid agonist and examined the effects of piracetam and naloxone on scopolamine-, ECS- and morphine-induced amnesia. The effects of ECS treatment and piracetam on morphine-induced analgesia were also examined. In these experiments, the drugs were administered either alone or in combination to animals and the effects were evaluated by using the elevated plus maze apparatus. The tail-flick test was performed to investigate the analgesic activities, and the rota-rod test was also included to examine piracetam action on the motor functions of mice.

MATERIALS AND METHODS

Mice of either sex weighing 20–25 g were obtained from the Animal House of Çukurova University. They were housed in metal cages in a laboratory maintained at a room temperature of 22°C with a 12-h light–dark cycle. Each cage had five animals of the same sex. Food and water were provided ad libitum. The principles of laboratory animal care published by NIH were followed during the experiments.

Elevated plus maze experiments

The plus maze consisted of two open arms, 10×50 cm, and two enclosed arms, $10 \times 50 \times 50$ cm [6]. The arms of each type, which were opposite each other, extended from a central platform (10×10 cm) raised 50 cm above the floor. The open arms and central platform were painted white and enclosed arms painted black. The principle in this experiment is based upon the aversive behaviour of rodents to open and high spaces. The animals dislike open and high spaces and move from them to an enclosed arm. The time before an animal enters an enclosed arm is termed the latency period (LP). Training (repeated exposure of animal to open arms) shortens this parameter, possibly as a consequence of learning acquisition and retention. In line with this observation, the measurements of LP values on the second day are significantly shorter compared with those on the first day and this continues over the subsequent days. Animals trained previously for two days were treated with scopolamine, ECS or morphine to produce amnesia. Piracetam and naloxone were used to test whether the amnestic situation is reversed by these agents.

Group I (control group)

The purpose of this experiment was to test whether training with the elevated plus maze of animals would cause the LP to shorten. Mice were individually placed at the end of one of the open arms facing the central platform and LP was recorded. Ten seconds after LP measurement, the animal was taken from the apparatus and put back in its own cage. The same test was repeated on subsequent days. One trial was performed each day on each mouse over 3–5 consecutive days. The time between training trials was 24 h. If the mouse did not enter the enclosed arms within 120 s, the LP was assigned to 120 s. The mice that were not able to shorten LP on the second day were not included in the experiments. No significant differences were observed between the LP values of the 3rd day and those of following days (Figure 1) and we thus chose 3 consecutive days for the assessment of LPs in the remaining experiments.

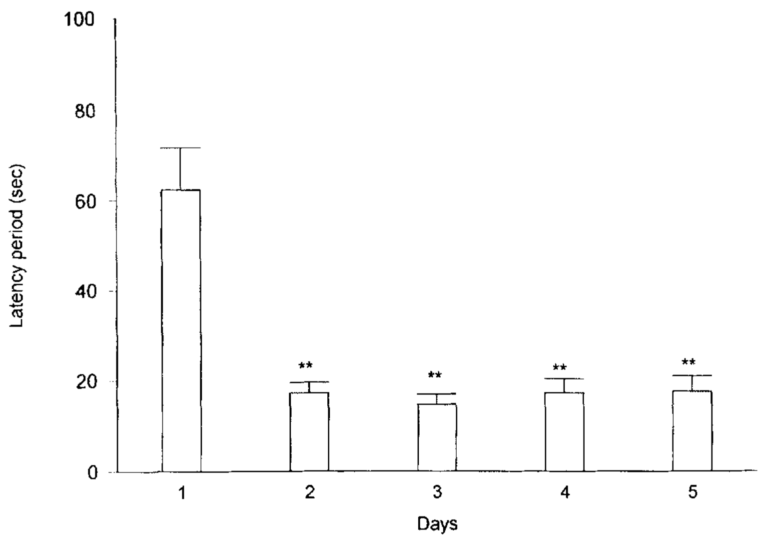

Figure 1. The latency periods of mice placed on the elevated plus maze for five consecutive days. $^{**}p < 0.01$: significantly different from the first day using Dunnett's test

Group II

The second group of experiments investigated the effects of scopolamine (0.1, 0.5, 1 or 2 mg/kg sc), ECS (5, 10, 15 or 20 mA; 1 s; 60 Hz) or morphine (0.5, 1, 2 or 4 μg icv) treatments on the LPs. In all cases, animals received scopolamine, ECS and morphine immediately after the plus maze trial on the second day. Twenty-four hours later (3rd day), the LPs were recorded once more as described before. Also, the effects of sham (receiving no ECS treatment) and control (taking vehicle) groups were separately established.

Group III

The third group of experiments examined the effect of piracetam (25, 50, 75, 100 or 200 mg/kg ip) on the trained animals that had been previously treated with scopolamine, ECS or morphine. Piracetam was administered 30 min before the plus maze trial of the second day; scopolamine (0.1 mg/kg sc), ECS (5 mA), or morphine (2 μg icv) were applied immediately after the plus maze trial. The procedure for LP measurements was the same as that described in the group I. Sham and control groups were also tested.

Group IV

The fourth group of experiments investigated the effect of naloxone (1 mg/kg ip) on the amnesia induced by scopolamine (0.1 mg/kg sc), ECS (5 mA) or morphine (2 μg icv). The procedure was identical to that described in the Group III experiments: like piracetam, naloxone was administered 30 min before the second trial with the plus maze.

Tail-flick experiments

The antinociceptive responses to ECS, morphine or ECS + morphine were measured using the radiant heat tail-flick test [9]. The lamp intensity was set up to provide a pre-drug response time of 2–4 s. A cut-off time of 10 s was used to prevent damage to the tail. The percent maximal possible effect (%MPE) was calculated for each mouse by using the formula shown in *Calculations and statistical analysis* below. The mean values were then calculated for each group and compared statistically.

Morphine was injected subcutaneously at doses of 0.50, 1, 2.5 or 5 mg/kg to mice. Animals received ECS (5 mA) 30 min after morphine injection and were immediately subjected to the tail-flick test. The aim of this test was to determine whether ECS causes an antinociceptive action. This seemed to be possible because there are some speculations about the possibility of ECS-induced opioid release in the brain [7,8]. To assess the effect of piracetam on morphine-induced analgesia, piracetam (25, 50, 75 or 100 mg/kg ip) were given 30 min before morphine injection (10 mg/kg sc). Tail-flick latency was measured 10 min after drug administration.

Rota-rod test

This was applied to examine the effect of piracetam (200 mg/kg ip) on motor functions. The mice receiving piracetam or saline were placed on the rotating bar (18 rpm) of the rota-rod apparatus for 5 min and dropping time was determined.

Calculations and statistical analysis

The mean LP values ($\pm$SE) of the first, second and third days were separately determined. Comparisons were made between LPs of the first or second day and that of the third day.

Percent mean changes

These values were considered established for comparisons of the LPs of first or second days with those of third days in a different way and calculated in the following way:

$$A = (\chi_2 + SE_2) \times 100/\chi_1 + SE_1$$
$$B = (\chi_2 - SE_2) \times 100/\chi_1 - SE_1$$
$$\%\ \text{mean change} = A + B/2$$

where χ_1 is the mean LP values of first or second days and χ_2 is the mean LP value of the third day.

The maximum possible effect for analgesia was calculated according to the following formula [10]:

$$\%MPE = (\text{postdrug time} - \text{predrug time}) \times 100/(10 - \text{predrug time})$$

Apart from % mean changes, results were expressed as the mean $\pm$ SE and analysed by means of Student's *t*-test (for comparison of two groups), ANOVA followed by Newman-Keul's test (for multiple comparison) or Dunnet's test (for comparison between multiple groups and one group).

Drugs

Scopolamine hydrobromide, morphine sulphate, piracetam and naloxone hydrochloride were purchased from Sigma Chemical Company and dissolved in 0.9% NaCl solution.

RESULTS

The latency period for control mice to move from the open arm to the enclosed arm was significantly shorter on the second day than that on the first day and remained so on the subsequent days (third, fourth and fifth days) as seen in Figure 1. Analysis of LPs by using Dunnett's test revealed a significant difference ($p < 0.01$) between the LP value of the 1st day and that of the 2nd day (Figure 1). The mean duration on the 3rd, 4th and 5th days was also found to be significantly different from that on the 1st day ($p < 0.01$) although there were no significant differences among those of the last 4 days.

TABLE 1
Various parameters of scopolamine-, ECS- and morphine-induced amnesia

	n	Latency period (s) Day 1	Day 2	Day 3	% Mean change	p values
Scopolamine						
0 mg/kg	14	38.6±1.89	19.4±1.62	17.7±1.21	91.35	–
0.1 mg/kg	14	39.2±3.60	23.1±2.62	48.6±5.66	210.32	<0.01
0.5 mg/kg	15	50.9±8.58	20.5±2.43	42.1±8.60	203.25	<0.05
1 mg/kg	16	54.8±9.86	16.6±1.19	47.3±10.2	281.99	<0.05
2 mg/kg	15	47.6±6.96	20.7±1.92	58.5±9.17	280.92	<0.01
ECS						
Sham	12	69.0±10.5	12.1±3.10	11.5±2.30	96.51	–
5 mA	16	40.7±2.90	21.9±1.70	48.9±4.02	223.21	<0.01
10 mA	16	47.0±7.60	26.1±3.50	51.9±10.9	196.79	<0.01
15 mA	14	34.0±2.61	18.1±1.65	30.6±4.32	168.29	<0.01
20 mA	10	33.5±3.18	16.1±2.07	31.1±4.04	193.13	<0.01
Morphine						
0 μg	10	60.1±13.3	15.8±2.48	11.8±2.63	73.86	–
0.5 μg	10	66.5±13.0	12.7±1.68	11.6±2.45	90.37	–
1 μg	10	66.1±12.7	15.9±2.39	44.5±10.1	276.00	<0.05
2 μg	12	87.0±9.96	28.9±7.44	91.8±8.52	332.07	<0.01
4 μg	10	56.2±9.65	23.5±5.24	32.9±10.9	136.36	–

% Mean changes were calculated by using the mean LP values of the second and third days according to the formula in the text; p values show the difference between the mean LP values of the second and third day according to ANOVA followed by Newman-Keul's test

Scopolamine (0.1, 0.5, 1 and 2 mg/kg sc) prolonged LPs on the third day (Table 1). Significant amnestic effects were determined over the range of scopolamine concentrations studied. As little as 0.1 mg/kg of scopolamine seemed enough to produce a remarkable amnestic effect and we thus chose it as a standard amnestic dose for the remaining experiments.

All ECS currents (5, 10, 15 and 20 mA; 1 s; 60 Hz) examined in our experiments prolonged LPs on the 3rd day (Table 1). These results indicate that this treatment results in amnesia. It was apparently most noticeable in animals receiving ECS treatment of 5 mA as confirmed by the figures recorded in Table 1. Following this current, no deaths occurred. Thus, a current of 5 mA was employed in the remaining experiments.

TABLE 2
Various parameters affected by piracetam on scopolamine-, ECS- and morphine-induced amnesia

Piracetam dose (mg/kg)	n	Latency period (s) Day 1	Day 2	Day 3	% Mean change	p values
Scopolamine–Piracetam						
0	14	39.9±3.47	21.6 ±2.11	48.1 ±5.93	115.8	–
25	12	35.1±2.11	16.1 ±1.39	13.2 ±1.37	37.5	<0.01
50	14	57.1±10.6	13.4 ±2.21	10.4 ±1.59	18.9	<0.01
100	12	46.3±12.7	8.58±1.44	7.08±1.36	15.7	<0.01
200	14	42.1±11.4	10.1 ±1.69	20.1 ±7.86	46.1	–
ECS–Piracetam						
0	12	47.2±4.92	22.3 ±2.65	41.2 ±2.32	87.75	–
25	12	41.4±4.09	19.0 ±1.65	44.9 ±9.20	107.3	–
50	14	34.9±1.94	15.8 ±1.25	13.9 ±1.27	39.8	<0.01
75	10	37.4±2.26	20.5 ±2.01	17.4 ±1.77	46.4	<0.01
100	14	40.1±2.17	20.5 ±1.48	24.9 ±2.58	61.9	<0.01
200	10	44.1±6.23	20.2 ±3.25	30.2 ±3.38	68.77	<0.05
Morphine–Piracetam						
0	12	87.0±9.96	28.9 ±7.44	91.8 ±8.52	105.8	–
25	10	70.5±12.6	19.0 ±5.28	52.9 ±16.1	73.3	–
50	10	62.2±12.6	17.3 ±2.35	18.3 ±3.33	23.5	<0.01
75	10	72.6±13.8	19.7 ±6.17	21.7 ±11.0	28.1	<0.01
100	10	98.2±10.7	21.9 ±3.72	23.5 ±11.4	23.0	<0.01
200	10	66.0±6.82	24.2 ±4.29	56.0 ±4.93	84.98	–

% Mean changes were calculated by using the mean LP values of the first and third days according to the formula in the text; p values, which were determined according to ANOVA followed by Newman-Keul's test, show the difference between the mean LP values of the first and third days

Morphine (0.5, 1, 2 or 4 μg icv) also elicited amnesia on the 3rd day, but these effects differed from those of scopolamine in having actions that clearly possessed a characteristic bell-shaped dose–response relationship in the dose range studied in these experiments (Table 1). The dose of morphine employed in subsequent experiments was 2 μg icv.

Piracetam (25, 50, 75, 100 or 200 mg/kg ip) prevented the prolongation of LPs induced by scopolamine (0.1 mg/kg) on the third day. It was most effective at 50 and 100 mg/kg (Table 2). Interestingly, 200 mg/kg of piracetam appeared to be less effective against scopolamine amnesia, a finding which explains the bell-shaped dose–response relationship. Except for minor differences, LP prolongations due to either ECS or

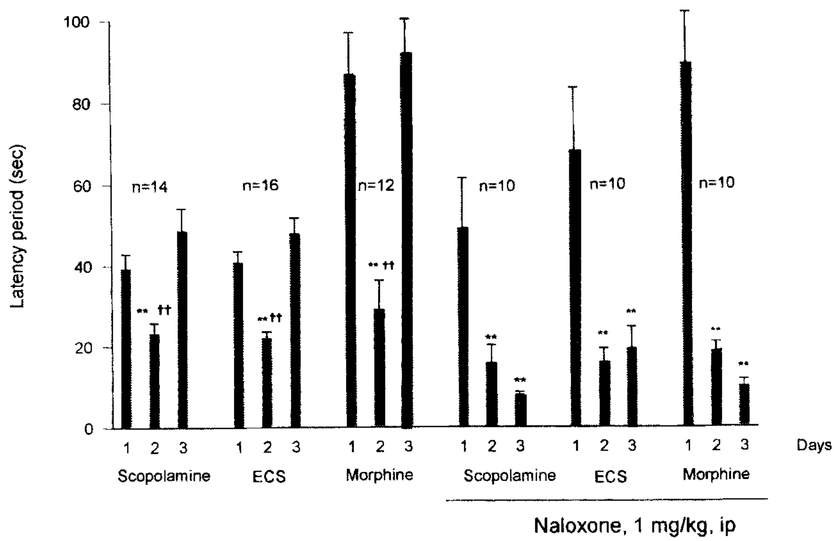

Figure 2. The effects of naloxone on the amnesia induced by scopolamine, ECS and morphine. $^{**}p<0.01$: significantly different from the first day; ††$p<0.01$: significantly different from the third day using ANOVA followed by the Newman-Keul's test

morphine responded to piracetam in a manner similar to that observed with scopolamine-induced LP prolongation (Table 2). These results suggest that the action of piracetam in reversing the amnesia tends to diminish as its dose is increased to 100 mg/kg or more, a finding which suggests a bell-shaped dose–response curve. However, this tendency seemed to be less marked in the ECS–piracetam interaction.

In order to investigate non-specific effects of piracetam, naive mice were treated with various doses of this substance. Piracetam itself failed to change LP values and also failed to affect the rota-rod performance of the mice. Data from these tests are not shown.

In the experiments examining the effect of naloxone, a narcotic antagonist, it was found that this substance (1 mg/kg ip) clearly prevented the LP prolongation induced by 0.1 mg/kg sc scopolamine, 5 mA ECS and 2 μg icv morphine as shown in Figure 2.

The effect of ECS on the analgesia is shown in Figure 3. ECS treatment itself elicited no analgesic activity but enhanced the analgesia caused by morphine (0.25, 1 and 5 mg/kg sc). The doses of morphine having no analgesic activity produced noticable analgesia when ECS and morphine were applied together. On the other hand, piracetam attenuated the morphine analgesia in animals tested with the tail-flick experiments (Figure 4).

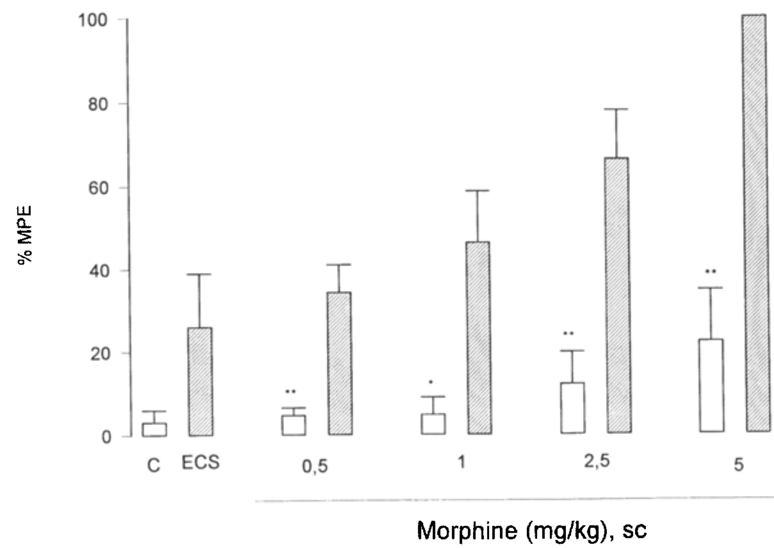

Figure 3. The effect of ECS treatment on morphine analgesia. Ten mice were used for each group. $*p<0.05$, $**p<0.01$: significantly different from the non-ECS-treated groups using Student's t-test

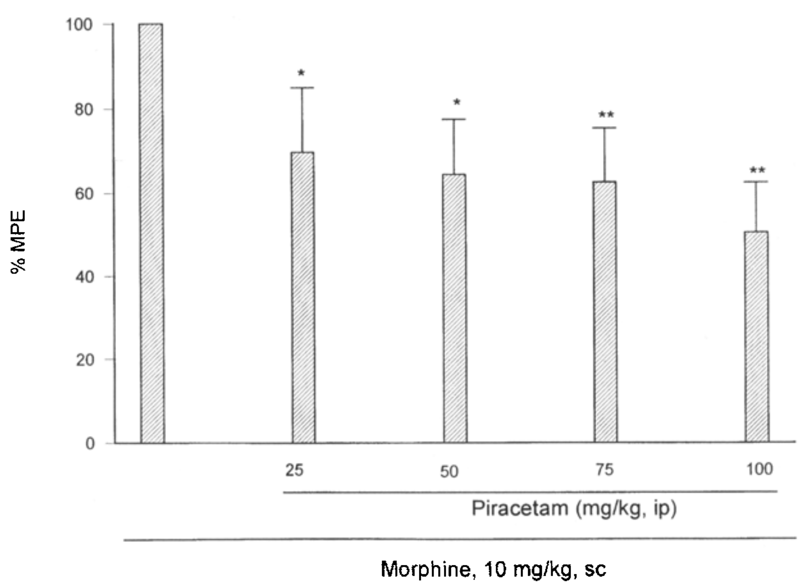

Figure 4. The effect of piracetam on morphine analgesia. Ten mice were used for each group. $*p<0.05$, $**p<0.01$: significantly different from the morphine group using Student's t-test

DISCUSSION

In the present study, the naive mice placed at the end of one of the open arms of the elevated plus maze usually entered one of the enclosed arms in 30–60 s on the first day. They entered an enclosed arm in a significantly shorter time when the procedure was repeated 24 h later. These observations, which are in good agreement with results reported previously [6,11–13], indicate that the mice had learned the safety region and then remembered it. Pellow and File [11] introduced the elevated plus maze for measurement of anxiety and Itoh et al. [6] first used it for evaluation of memory. The latter authors discussed the advantages of the elevated plus maze over other memory tests, such as passive–active avoidance, radial, Y, T or the water maze, in their studies [6].

We have shown that scopolamine, ECS, or morphine produced amnesia, increasing the latency periods of mice on the third day. The amnestic effects of these agents have been demonstrated in many other studies using other memory-measuring methods [4,14–19]. Scopolamine appeared to be the most useful amnestic agent, as indicated by many other studies [14–16], and thus we used it as a standard amnestic substance to confirm the utility of our method. In contrast to other studies mentioned above, scopolamine, over a wide range of dosages, exerted nearly equivalent effects on learning and memory in these experiments.

The effect of ECS on the behaviour of mice placed in the plus maze was the same as that seen with scopolamine. All the electric currents we used produced amnesia in mice. However, the high currents (15 and 20 mA) produced some convulsions and deaths in a few animals, so we used the lowest degree of electric current (5 mA) in our experiments. ECS-induced amnesia could result from the activation of more than one mechanism since this treatment results in some changes in the functions of the central nervous system, such as a decrease in Ach level [17], increase in acetylcholinesterase activity [20] and release of massive amount of brain endorphine and enkephaline [21]. Repeated administration of ECS produced a significant enhancement of baclofen-induced inhibition of 5-HT release, possibly by interaction with the function of $(GABA)_B$ receptor [22]. Taken together, these suggest that ECS-induced amnesia seems to be a complicated phenomenon and this makes it difficult to interpret drug actions. The data obtained from morphine-treated animals indicate that this substance produces an impairment of the cognitive functions resulting in amnesia, in agreement with previous results [18,19]. An interesting aspect of the dose–response relationships we observed here is that the response to a morphine dose as high as 4 μg is smaller than the responses to 1 and 2 μg, and is not much different from that to 0.5 μg; a characteristic reminiscent of the bell-shaped dose–response relationship, which was previously demonstrated as a property of naloxone-induced antiamnesia in mice [23].

Piracetam attenuated the amnesia induced by scopolamine, ECS and morphine. Significant p values were obtained when LPs for the first day were compared with those for the third day. In agreement with this, the same substance was reported to ameliorate the cognitive impairment in three models of amnesia (scopolamine-, diazepam- and ECS-induced amnesia) in mice [24]. Similarly, % mean changes follow a similar pattern. The more effective the reversion, the smaller the value of % mean change. When animals were subjected to 200 mg piracetam, LP values increased and % mean response became larger. The latter result is in accordance with the finding that the effects of the drug which improve cognitive functions are not dose dependent and are inverted U shaped or bell shaped [25].

The mechanism of the antiamnestic effect of piracetam has been discussed in many studies [for review see Ref. 1]. It seems that piracetam affects a large variety of neurotransmission systems. In this study, we focused our attention on the piracetam action against ECS-induced amnesia. ECS applications induce some changes in the functions of CNS by affecting various neurotransmitter systems, such as cholinergic, GABAergic, noradrenergic, serotonergic and opiatergic transmissions. It is impossible to say that the antiamnestic effect of piracetam is due to one of these changes. For example, the ECS-induced decrease in the Ach content in cortex and hippocampus was unaffected by piracetam while the amnesia was attenuated [17]. On the other hand, piracetam has no significant effect on GABA receptors, and does not affect the synaptosomal uptake and GABA levels in either brain or plasma where ECS inhibits GABA synthesis [26–28]. Conversely, the physostigmine effect improved the amnesia induced by scopolamine but not by ECS [6]. All these results directed us to the relationship between the ECS-induced amnesia and the opiatergic system. In recent years much attention has been devoted in the literature to the β-endorphine release after ECS treatment [7,8,21]. The potentialization of morphine analgesia by ECS in this study supports the idea that ECS causes endogeneous opioids to be released in the brain. The inhibitory effect of piracetam on ECS-induced amnesia may be partly due to an interaction between piracetam and the β-endorphine released after ECS. In an earlier study, it was stated that prolonged consumption of piracetam results in a three-fold decrease in β-endorphine concentrations in plasma and an increase in cAMP content in rats [29]. Other experimental evidence supporting the suggestion that there may be an effect of piracetam on the central opiatergic system arises from the finding that this nootropic can attenuate the morphine analgesia apparently in a dose-dependent manner. Finally, the antiamnestic effect of naloxone which has significantly improved all experimental amnesias in this study, indicates an important role of opioid receptor blockade on the modulation of memory.

In conclusion, the data obtained in this study imply that the opiatergic system plays an important role in the mediation of the nootropic action of piracetam.

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