Pharmacology Biochemistry and Behavior, Vol. 59, No. 3, pp. 685–689, 1998

0091-3057/98 $19.00 + .00

ELSEVIER

PII S0091-3057(97)00462-0

The Effect of Baclofen and AP-7 on Selected Behavior in Rats

H. CAR AND K. WIŚNIEWSKI

Department of Pharmacology, Medical Academy in Białystok, Białystok, Poland

Received 8 January 1997; Revised 4 August 1997; Accepted 4 August 1997

CAR, H. AND K. WIZŚNIEWSKI. *The effect of baclofen and AP-7 on selected behavior in rats.* PHARMACOL BIOCHEM BEHAV **59**(3) 685–689, 1998.—Synaptic plasticity, cognitive performance, learning, and memory appear to be determined by the balance between GABAergic inhibitory and glutaminergic excitatory amino acids (EAA). To evaluate this role of amino acids the effects of baclofen (0.5 mg/kg IP), GABA-B receptor agonist and AP-7 (5 nmol ICV)–NMDA (*N*-metyl-D-aspartate) receptor antagonist on the processes of retrieval, consolidation of conditioned reflexes, object recognition, and locomotor activity were tested in rats. Neither AP-7 nor baclofen alone changed locomotor activity, but coadministration of AP-7 and baclofen significantly decreased this activity in the open-field test. Neither AP-7 nor baclofen influenced retrieval or consolidation in the passive avoidance situation when administred alone. Significantly prolonged retrieval and consolidation were observed when AP-7 and baclofen were given together. We did not find differences in effects of either AP-7 or baclofen on object recognition, regardless whether administered alone or in combination. © 1998 Elsevier Science Inc.

AP-7 Baclofen Behavioral tests Rat

TWO distinct types of amino acids can mediate synaptic transmission in the central neurvous system: inhibitory (GABAergic) and excitatory glutaminergic (EAA) (20).

Research indicates interaction between these two systems. Metabolic interaction: tight relationship between GABA and glutamate biosyntesis, uptake systems (17), GABA can inhibit the neuronal release of L-glutamate (23).

Electrophysiological interaction: inhibitory postsynaptic potentials (IPSPs) mediated by GABA receptors are followed by excitatory postsynaptic potentials (EPSPs) mediated by glutaminergic receptors (14), administration of GABA or baclofen also depresses EPSPs and IPSPs in the hippocampus via presynaptic receptors (19) and GABA and EAA are important in induction of LTP (long-term potentiation) (3,5). All these effects are mediated by receptors of GABAergic and glutaminergic systems. Some structural similarities between the two receptors types have been found (6).

In previous research we attempted to find interaction between activation of GABA-A receptors and inhibition of NMDA receptors in rats. In our studies we used behavioral tests. No interaction was found (unpublished data).

In the present study we tested whether activation of GABA-B receptors by agonist baclofen-influenced effects of selective, competitive antagonist of NMDA receptors—DL-2-amino-7-phosphono-heptanoate (AP-7).

METHOD

Animals

Subjects were white, male Wistar rats weighing 160–180 g. The animals were fed standard diet and housed in group cages in an air-conditioned room with a 12 L:12 D cycle beginning at 0700 h. All experiments were carried out between 0800 and 1200 h.

Drug Administration

DL-2-Amino-7-phosphono-heptanoic acid (AP-7), Sigma, was administered into the lateral ventricle of the brain (ICV) (10) in a dose 5 nmol/10 μl (22). Two days before behavioral tests, a burr hole of 0.5 mm in diameter was drilled in the rat's skull, 2.5 mm laterally and 1 mm caudally from the point of intersection of bregma and the superior sagittal suture on the right side of the head. Ether anesthesia was used.

ICV injection was made to a depth of 4.5 mm with a Hamilton microsyringe. After termination of each experi-

Requests for reprints should be addressed to Professor Konstanty Wiśniewski, Department of Pharmacology, Medical Academy in Białystok, 2c Mickiewicza, 15–222 Białystok, Poland.

ment, all animals were killed by decapitation, their brains were removed, and the site of injection was verified macroscopically. Animals with inappropriate injection sites were not considered for analysis.

(−) Baclofen, Polfa Starogard, was administered intraperitonelly (IP) in a dose 0.5 mg/kg (18).

Saline (0.9% NaCl) (Polfa Poznań) was administered ICV in a volume of 5 μl or IP in a dose of 0.1 ml/kg (10).

Behavioral Tests

Object recognition. Apparatus: a plastic box 62 cm long, 38 cm wide, and 20 cm high covered with a wire mesh lid was used. Objects for discrimination test were made of glass or porcelain and existed in duplicate. Apparently they had no natural significance for rats and they have never been associated with reinforcement. Their weight was such that they could not be displaced by rats.

The experiments were conducted in the same room as passive avoidance test as well as locomotor and exploratory activity tests.

Behavioral testing: the procedure was similar to that described by Ennaceur and Delacour (9) and Cavoy and Delacour (4) with some modifications by Braszko (2). It may be summarized as follows: all rats were submitted to two habituation sessions at an interval of 60 min, in which they were allowed 3 min to explore the apparatus. Twenty-four hours later testing began. Each session was made of two trials each lasting 3 min and separated by a 60-min period. In the first trial (T1) rats were exposed to two identical objects that constituted samples "a" and "b" placed in the two back corners of the box. The time of each object exploration by the rats was recorded. Immediately after T1 the rat was removed, and received one of the following: saline ($n = 13$), AP-7 ($n = 15$), baclofen ($n = 15$), or a combination: AP-7 + baclofen ($n = 13$) as an ICV or IP injection. After 60 min rats were returned to the apparatus. In the second trial (T2) rats were exposed to two objects that were introduced into the apparatus: the familiar object "a" and a new object "b" placed in the same positions as during T1. The object "a" presented during T2 was a duplicate of the sample presented in T1 to avoid olfactory trails. For each animal, the role (familiarity or new object) as well as the relative position of the two objects were randomly permuted.

The time of each object exploration was recorded. Exploration of an object was defined as touching it with the nose. Turning around or sitting on the object was not considered as exploratory behavior. From this measure the following variables were defined: A—the time spent on exploring object "a" and "b" in T1; A′ and B—the time spent on exploring the familiar and the new object in T2, respectively.

Object recognition was measured by the B-A′ variable (discrimination beetwen the new object "b" and the familiar one "a"). As this time may be biased by differences in overall levels of exploration, the total exploration (in T2 by B + A′ and B − A′/B + A′) were also computed.

Passive avoidance response training. The response was induced using the one-trial-learning method of Ader (1). The apparatus consisted of a 6 × 25 cm platform illuminated with a 25 W electric bulb connected through a 6 × 6 cm opening with a dark compartment (40 × 40 × 40 cm). The floor of the cage was made of metal rods 3 mm in diameter, spaced at 1 cm. The investigation took advantage of the natural preference of rats to stay in dark compartments. The test lasted 3 days. On the first day, after 2 min of habituation in the dark compartment, they were immediately removed. Two similar trials, at an interval of 2 min, were carried out on the second day. After the first trial rats were allowed to stay in the dark compartment for 10–15 s. In the second trial when a rat entered the dark compartment it received a foot shock (0.25 mA, 3 s) delivered through the metal rods. The presence of the passive avoidance was checked 24 h later. Rats were placed on the illuminated platform once more and the latency to enter the dark compartment was measured, with the cut time of 300 s. To determine their effect on consolidation, according to the protocol proposed by Matthies (13), AP-7 and baclofen were administered on the second day immediately after the completion of induction of passive avoidance. To determine their effect on retrieval, the substances were administered on the third day, 30 min before the test for preservation of the passive avoidance response.

Locomotor and exploratory activity. The open-field test was used for estimation of locomotor activity of rats. The apparatus consisted of a square of 100 × 100 cm white floor, which was divided by 8 lines into 25 equal squares, and surrounded by white walls, 47 cm high. Four plastic bars, 20 cm high, were located in four different line crossings in the central area of the floor. A single rat was placed inside the apparatus for 1 min of adaptation. Subsequently, crossings, rearings, and bar approaches were counted manually for 5 min. AP-7 and baclofen were administered 30 min before the test.

Statistical Analysis

The statistical significance of the results was computed by analysis of variance (ANOVA) followed by modified *t*-statistics (21) when multiple means were to be compared.

RESULTS

The Effect of AP-7, Baclofen, and AP-7 With Baclofen on Object Recognition in Rats (Table 1)

The time spent on exploring object "a" in T1 (variable A) was comparable in all groups. Object recognition memory measure (B − A′) was not significantly different between the groups. Comparisons made with the ANOVA test revealed a weaker object recognition memory in rats treated with baclofen and with AP-7 alone compared with the saline, or AP-7 with baclofen-treated groups. The total time spent on exploring object "b" and "a"; in T2 (B + A′) was decreased in baclofen-treated rats. Changes in the B − A′/B + A′ variable in AP-7 and baclofen-treated rats were comparable. Value of this variable, however, was lower than in saline and AP-7 with baclofen-treated rats. The tendencies were not significant.

The Effect of AP-7, Baclofen, and AP-7 With Baclofen on Retrieval of Passive Avoidance in Rats (Fig. 1)

Neither AP-7 in a dose of 5 nmol (ICV) nor baclofen (0.5 mg/kg IP) changed the latency in rats. Coadministration of AP-7 and baclofen significantly prolonged the latency to enter the dark compartment.

The Effect of AP-7, Baclofen, and AP-7 With Baclofen on Consolidation of Passive Avoidance in Rats (Fig. 2)

Neither AP-7 nor baclofen changed the latency in rats, i.e., they did not change consolidation of passive avoidance, but coadministration of AP-7 with baclofen prolonged the time spent on the platform, i.e., enhanced consolidation.

TABLE 1

Object Recognition				
	Treatment			
Variables	Saline	AP7	Baclofen	AP-7+Baclofen
B-A′	1.73 (1.12)	−0.083 (0.67)	−0.875 (0.44)	1.75 (1.43)
A	15.45 (2.59)	18.83 (2.59)	14.125 (2.79)	14.5 (3.5)
B+A′	8.45 (2.23)	9.42 (2.38)	0.87 (0.44)	9 (1.92)
B-A′/B+A′	0.149 (0.11)	−1.152 (0.1)	−0.5 (0.26)	0.07 (0.22)

The rats were treated ICV with 5 nmol of AP-7, and IP 0.5 mg/kg of baclofen, or ICV and IP saline. See text for further details. Variables (in seconds) describing object recognition (see text). Values are means from 13–15 subjects and ±SEM (in parentheses).

The Effect of AP-7, Baclofen, and AP-7 With Baclofen on Locomotor and Exploratory Activity of Rats in the Open-Field Test (Fig. 3)

Neither AP-7 nor baclofen produced significant changes estimated on the basis of the number of crossed fields, rearings, and bar approaches throughout the observation period. Significant decrease in mobility was observed in rats treated with both AP-7 and baclofen.

DISCUSSION

In the present experiments, stimulation of the GABA-B receptor by baclofen (0.5 mg/kg IP) did not change locomotor activity in the open-field test.

Similar result was obtained after inhibition of the NMDA receptor by AP-7. Baclofen and AP-7, when used in combination, caused reduction of mobility in rats. Baclofen is a widely used antispastic drug and its effect is due to influence on

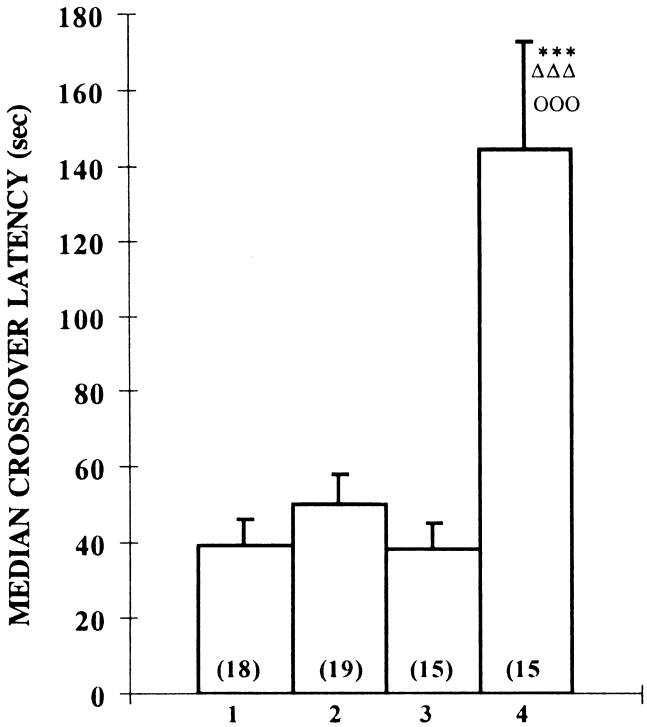

FIG. 1. The effect of AP-7, baclofen, and AP-7 with baclofen on retrieval in the passive avoidance situation in rats. Columns represent means ± SEM of the number of animals indicated in the columns. 1) Saline 5 μl ICV and saline 0.1 ml/100 g IP (39.67 ± 6.55); 2) AP-7 5 nmol ICV and saline IP (50.47 ± 7.4); 3) saline ICV and baclofen 0.5 mg/kg IP (38.73 ± 6.52); 4) AP-7 ICV and baclofen IP (144.53 ± 28.46). ***p(1–4) < 0.001,ΔΔΔp(2–4) < 0.001, OOOp(3–4) < 0.001, $F(3, 63) = 12.02$, $p < 0.001$.

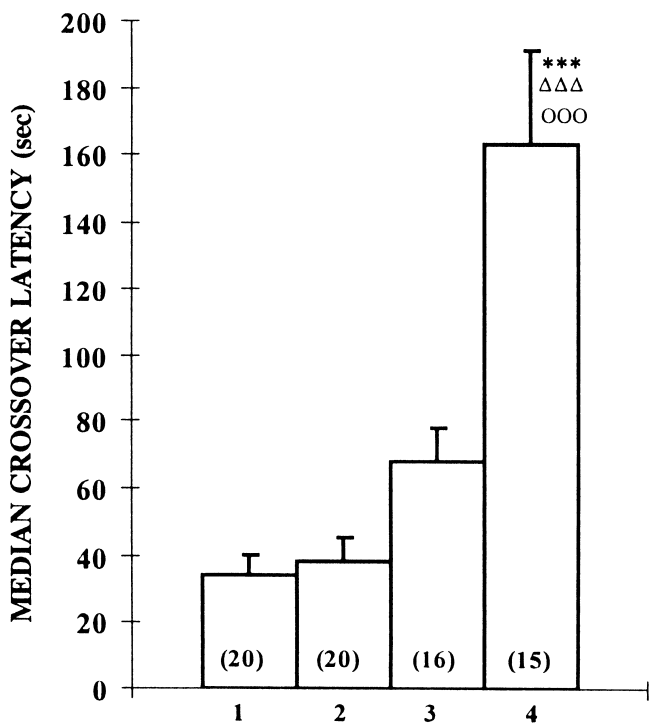

FIG. 2. The effect of AP-7, baclofen, and AP-7 with baclofen on consolidation in the passive avoidance situation in rats. Columns represent means ± SEM of the number of animals indicated in the columns. 1) Saline 5 μl ICV and saline 0.1 ml/100 g IP (34.35 ± 6.24); 2) AP-7 5 nmol ICV and saline IP (38.65 ± 7.05); 3) saline ICV and baclofen 0.5 mg/kg IP (68.0 ± 10.05); 4) AP-7 ICV and baclofen IP (163.25 ± 27.95). ***p(1–4) < 0.001, ΔΔΔp(2–4) < 0.001, OOOp(3–4) < 0.001, $F(3, 67) = 11.9$, $p < 0.001$.

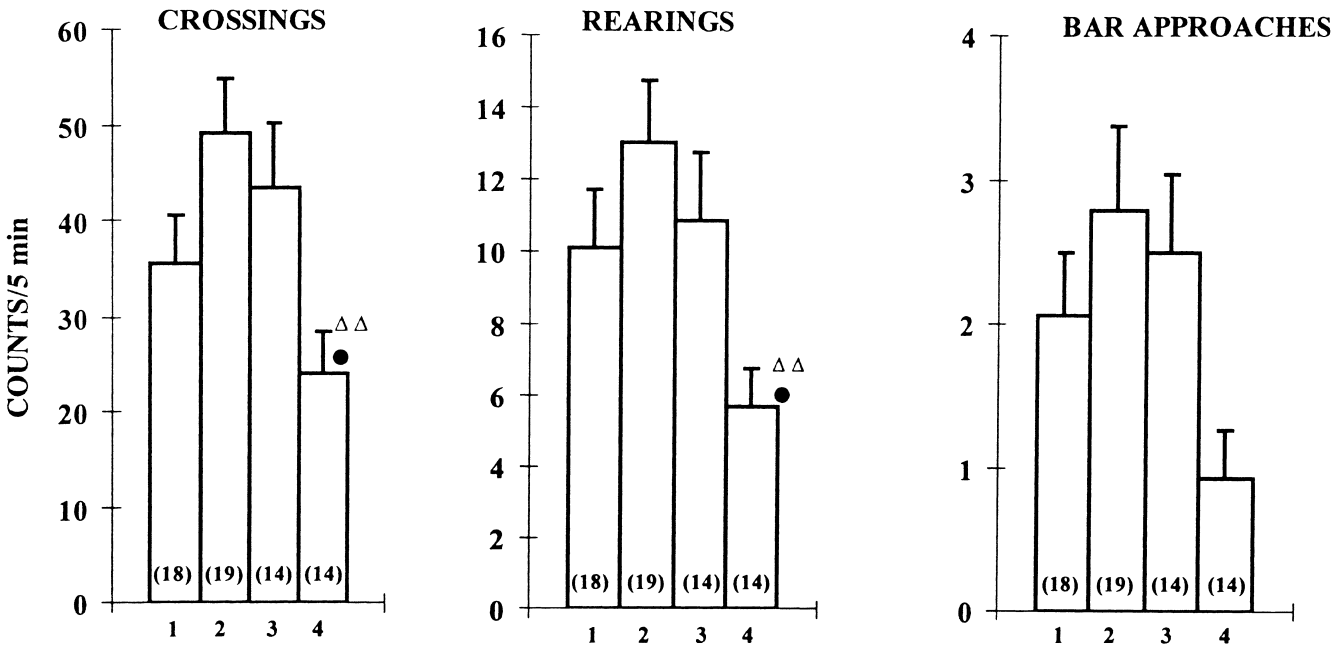

FIG. 3. The effect of AP-7, baclofen, and AP-7 with baclofen on the number of crossings, rearings, and bar approaches in the open field. Columns represent means ± SEM of the number of animals indicated in the columns. 1) saline 5 μl ICV and saline 0.1 ml/100 g IP, crossings (35.55 ± 4.98), rearings (10.11 ± 1.63), bar approaches (2.05 ± 0.43); 2) AP-7 5 nmol ICV and saline IP, crossings (49.09 ± 5.7), rearings (13.52 ± 1.73), bar approaches (2.78 ± 0.58); 3) saline ICV and baclofen 0.5 mg/kg IP, crossings (43.42 ± 6.69), rearings (10.85 ± 1.89), bar approaches (2.5 ± 0.53); 4) AP-7 ICV and baclofen IP, crossings (24.0 ± 4.54), rearings (5.64 ± 1.07), bar approaches (0.92 ± 0.33). △△p(2–4) < 0.001, ●p(3–4) < 0.05; crossings, F(3. 61) = 6.81, NS; rearings F(3. 61) = 3.79, $p < 0.02$; bar approaches, F(3. 61) = 2.49, NS.

GABA-B receptors (12) causing reduction of glutamate release in the spinal cord (14). On the other hand, a biphasic action of GABA agonist (muscimol, progabide, baclofen) can be observed: potentiation of behavior related to nigrostriatal dopamine neuron activity at very low doses of agonists, in contrast to the inhibition of dopamine neuron activity and receptor-mediated behavior at somewhat higher doses (12). AP-7, the competitive NMDA receptor antagonist, produces weak increases of dopamine turnover in limbic structures and can enhance locomotor activity (7,8,11).

Literature data indicate that baclofen impairs memory in humans (16). When injected (10 mg/kg IP) immediately after training it impaired posttraining of inhibitory avoidance in rats (3). In our experiments, we observed no effect of baclofen on retrieval in passive avoidance situation. We used a low dose of baclofen (0.5 mg/kg IP), and injection was given on the third day, before a single trial of retrieval.

In addition, baclofen, a selective GABA-B receptor agonist, has a pre- and postsynaptic effect. Presynaptically it reduces the release of excitatory and inhibitory transmitters (14). Moreover, baclofen can produce dose-dependent antinociception, reduces muscle tone, and may be involved in antidepressant action (20). All these effects can contribute to the retrieval in passive avoidance test and influence results.

Similarily, no change of retrieval in this test was obtained after administration of AP-7. The passive avoidance procedure was used to study the disruptive effects of glutamate antagonists on learning (7). NMDA receptor antagonists given systemically impair retention only if given before training, and not if administered even shortly afterwards (7,8,15). It is possible that the dose of 5 nmol of AP-7 given intracerebroventricularily and time of injection on the third day of training before measuring of retention did not influence this parameter of passive avoidance situation.

Unfortunately the nonspecific (nonassociative) effects of the NMDA receptor antagonist on learning is difficult to eliminate (15). Anxiolytic effects also seen with NMDA receptor antagonist are responsible for retention changes (15). In our study we obtained the facilitation of retrieval in passive avoidance test after administration of AP-7 and baclofen together.

First, we can notice a connection between the reduction of locomotor activity in the open-field test after coadministration of AP-7 and baclofen, and prolonged time of retrieval in the passive avoidance in the same groups of rats. Secondly, AP-7 and baclofen given together in one rat can decrease the level of glutamate in synapses.

On the other hand, the nonspecific effects of AP-7 can play a role (14). Administration of AP-7 or baclofen immediately after training on the second day (13) can influence consolidation in the passive avoidance situation. Similar to the retrieval process, we did not observe changes of consolidation after using AP-7 or baclofen. Literature data give no clear indication that NMDA or GABA-B receptors are involved in memory consolidation (7,8,15).

The enhancement of consolidation in the passive avoidance test, after coadministration of AP-7 and baclofen, was unexpected. The time of injection of these substances during the test (see above) excludes the possibility of their influence on locomotor activity as well as any anxiolytic effects. Probably these "beneficial" results depend on changes in concentration of glutamate after using AP-7 and baclofen (14).

The intensification of consolidation is the main point of interaction between AP-7 and baclofen. Unfortunately, we do not have sufficient data to explain this result.

The object recognition test was used to estimate the working memory (9). In the present study we did not observe changes in object recognition in rats after administration of AP-7 and baclofen either alone or in combination.

Pharmacological studies on the influence of NMDA receptor antagonist on working memory are rather confusing. In most studies, specific impairing effect of NMDA receptor antagonists on this kind of memory is restricted to a narrow dose range and is easily confounded by nonassociative factors (8). To our knowledge, there are no data on the effects of the GABA receptor agonist on working memory.

These results support our hypothesis about an interaction between the GABAergic and the glutaminergic systems. The main conclusion is that baclofen as a GABA-B receptor agonist modulates the effects of AP-7, selective NMDA receptor antagonist, on locomotor activity in the open-field test and on retrieval and consolidation in the passive avoidance test. However, more specific studies are needed to determine the precise mechanism of effects of baclofen and AP-7 on memory and learning processes.

ACKNOWLEDGEMENTS

The authors thank M. Kuziemko-Łęska, J. Ropelewska, E. Piotrowska, and Ł. Stalenczyk for technical assistance.

REFERENCES

1. Ader, R.; Weijnen, J. A. W. M.; Maleman, P.: Retention of a passive avoidance responses as a function of the intensity and duration of electric shock. Psychonom. Sci. 26:125–132; 1972.
2. Braszko, J. J.; Kułakowska, A.; Wiśniewski, K.: Angiotensin II and its fragment improve recognition but not spatial memory in rats. Brain Res. Bull. 37:627–631; 1995.
3. Brioni, J. D.: Role of GABA during the multiple consolidation of memory. Drug Dev. Res. 28:3–27; 1993.
4. Cavoy, A.; Delacour, J.: Spatial but not object recognition is impaired by aging in rats. Physiol. Behav. 53:527–530; 1993.
5. Collingridge, G. L.; Singer, W.: Excitatory amino acid receptors and synaptic plasticity. Trends Pharmacol. Sci. 11:290–296; 1990.
6. Costa, E.: Reminiscences and perspectives about research on neurotransmitter amino acids. In: Meldrum, S.; Moroni, F.; Simon, R. P.; Woods, J. H. Excitatory amino acids. New York: Raven Press Ltd; 1991:3–11.
7. Danysz, W.; Wróblewski, J. T.; Costa, E.: Learning impairment in rats by N-methyl-D-aspartate receptor antagonists. Neuropharmacology 27:653–656; 1988.
8. Danysz, W.; Zajączkowski, W.; Parsons, C. G.: Modulation of learning processes by ionotropic glutamate receptor ligands. Behav. Pharmacol. 6:455–474; 1995.
9. Ennaceur, A.; Delacour, J.: Effect of combined or separate administration of piracetam and choline on learning and memory in the rat. Psychopharmacology (Berlin) 92:58–67; 1987.
10. Herman, Z. S.: The effect of noradrenaline on rats behaviour. Psychopharmacology (Berlin) 16:396–374; 1970.
11. Hiramatsu, M.; Cho, A. K.; Nabeshima, T.: Comparison of the behavioural and biochemical effects of the NMDA receptor antagonist, MK-801 and phencyclidyne. Eur. J. Pharmacol. 166: 359–366;1989.
12. Lloyd, K. G.; Morselli, P. L.: Psychopharmacology of GABAergic grugs. In: Meltzer, H. Y., ed. Psychopharmacology. The third generation of progress. New York: Raven Press; 1987:183–195.
13. Matthies, H.: Pharmacology of learning and memory. Trends. Biochem. Sci. 1:333–337; 1980.
14. Misgeld, U.; Bijak, M.; Jarolimek, W.: A physiological role for GABA-B receptors and the effects of baclofen in the mammalian central nervous system. Prog. Neurobiol. 46:423–462; 1995.
15. Mondadori, C.; Weiskrantz, L.; Buerki, H.; Petschke, F.; Fagg, G. E.: NMDA receptor antagonists can enhance or impair learning performance in animals. Exp. Brain. Res. 75:449–456; 1989.
16. Sandyk, R.; Gilman, M. A.: Baclofen-induced memory impairment. Clin. Neuropharmacol. 8:294–295; 1985.
17. Simantov, R.: γ-Aminobutyric acid (GABA) enhances glutamate cytotoxicity in a cerebellar cell line. Brain Res. Bull. 24:711–715; 1990.
18. Shephard, R. A.; Wedlock, P.; Wilson, E.: Direct evidence for mediation of an anticonflict effect of baclofen by GABA-B receptors. Pharmacol. Biochem. Behav. 41:651–653; 1992.
19. Thompson, S.; Gähwiler, B.: Comparison of the actions of baclofen at pre- and postsynaptic receptors in the rat hippocampus in vitro. J. Physiol. 451: 329–345; 1992.
20. Turgeon, S. M.; Albin, R. L.: Pharmacology, distribution, cellular localization, and development of GABA-B binding in rodent cerebellum. Neuroscience 55:311–323; 1993.
21. Wallenstein, S.; Zucker, C. L.; Fliss, J. L.: Some statistical methods useful in circulation research. Circ. Res. 47:1–9; 1980.
22. Wiśniewski, K.; Artemowicz, B.; Lutostańska, A.: The estimation of interactions between arginine vasopressin and NMDA receptors in memory and learning processes. Acta Physiologica Hungerica (submitted).
23. Yu, O.; Chuang, D.-M.: Long-term GABA treatment elicits supersensitivity of quiqualate-prefering metabotropic glutamate receptor in cultured rat cerebellar neurons. J. Neurochem. 61:430–435; 1993.