Pharmacology Biochemistry and Behavior, Vol. 56, No. 4, pp. 583–587, 1997
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0091-3057/97 $17.00 + .00

PII S0091-3057(96)00406-6

Effects of Flunarizine and Nitrendipine on Electroconvulsive Shock- and Clonidine-Induced Amnesia

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Received 1 August 1995; Revised 10 June 1996; Accepted 21 June 1996

GENKOVA–PAPAZOVA, M., B. P. PETKOVA, M. LAZAROVA–BAKAROVA, E. BOYANOVA AND D. STANEVA–STOYTCHEVA. *Effects of flunarizine and nitrendipine on electroconvulsive shock- and clonidine-induced amnesia.* PHARMACOL BIOCHEM BEHAV **56**(4) 583–587, 1997.—This study was designed to examine the calcium channel blockers flunarizine and nitrendipine for their ability to prevent electroconvulsive shock (ECS)- or clonidine-induced deterioration of the inhibitory avoidance performance (step-down) in rats. Flunarizine (10 mg/kg) and nitrendipine (40 mg/kg) were found to prevent the ECS- or clonidine-provoked amnesia after oral administration for 12 days. The mechanisms of action of the two drugs are considered. The results of this study further suggest that calcium antagonists might be useful in the treatment of cognitive disorders. © 1997 Elsevier Science Inc.

Calcium channel blockers Flunarizine Nitrendipine Amnesia Electroconvulsive shock Clonidine Memory Inhibitory avoidance Rats

IN the search for drugs with protective effects against memory impairments provoked by aging and brain damage, calcium antagonists have gained increasing attention. Calcium channel blockers are widely used for the treatment of hypertension, but their pharmacological characteristics suggest the efficiency of these drugs in the therapy of various central nervous system disorders (40). This possibility has been verified by animal models of epilepsy, cerebral ischemia, depression, and dementia (1,19). However, little is now known of the cognitive effects of calcium antagonists. Only nimodipine has been thoroughly studied with respect to its effects on memory (37). Some authors claim that calcium antagonists significantly reduce retention (7,33), whereas others have shown improved acquisition and enhanced retention after nimodipine (14,23,24,32), nifedipine (45), and clentiazem (26). Saha et al. (36) observed that diltiazem antagonized the effects of ketamine on acquisition and memory consolidation in step-down trained mice. The lack of data about the effects of calcium antagonists on experimentally induced amnesia stimulated us to study the influence of two classes of calcium channel blockers on amnesia provoked by electroconvulsive shock (ECS) or by the α_2-adrenoceptor agonist clonidine in step-down trained rats. ECS and clonidine at low doses impair memory consolidation in different behavioral tasks (39). Calcium is essential for both initiation and propagation of seizure discharges as well as for the signal transduction after α-adrenergic receptor stimulation. α_2-Adrenoceptors are suggested to regulate Ca^{2+} influx (42). Disturbances in calcium homeostasis might be considered a mechanism underlying the two types of amnesia.

METHOD

Subjects

Male Wistar rats weighing 180–200 g were used. Animals (160) were housed in plastic cages (80 × 40 × 10 cm; 10 rats per experimental group) in a room maintained at constant temperature and on a 12 h light/12 h dark cycle (lights on from 0700 to 1900). Food and water were provided ad lib except during training and testing.

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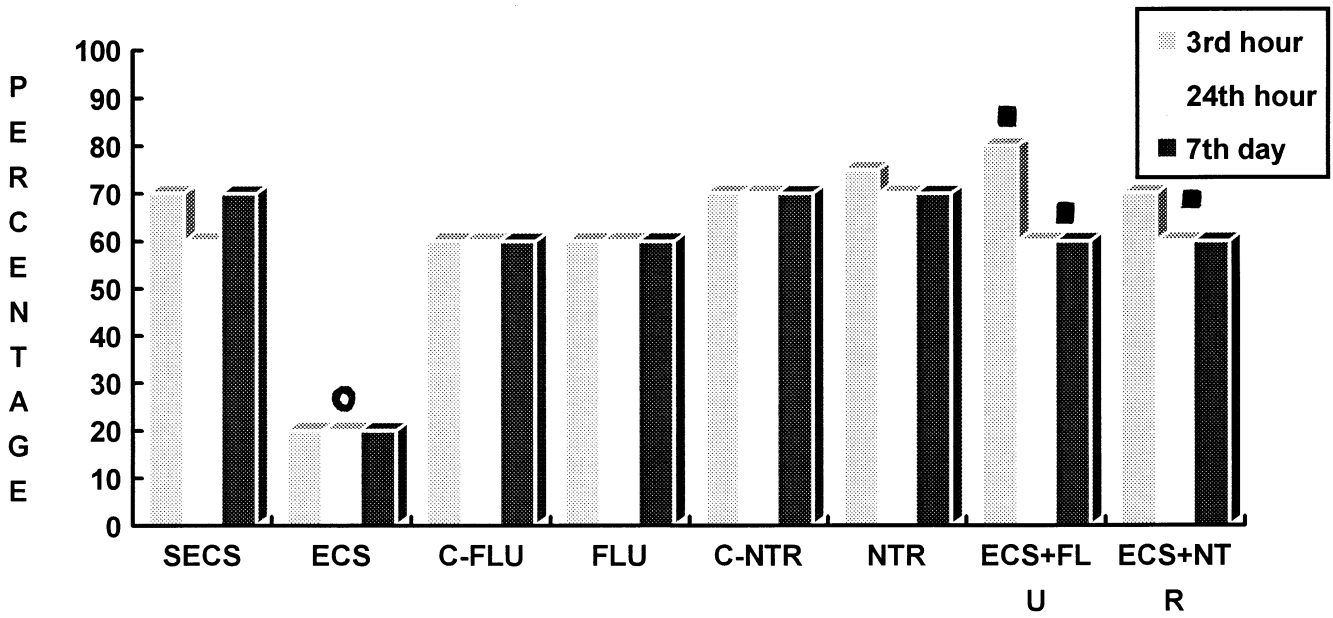

FIG. 1. Effects of flunarizine and nitrendipine on ECS-induced memory disturbances in step-down passive avoidance in rats ($n = 10$). On the ordinate: percentage of rats attaining the learning criterion; on the abscissa: SECS, sham ECS; ECS, electroconvulsive shock; C-FLU, controls treated with the vehicle of flunarizine; FLU, flunarizine (10 mg/kg); C-NTR, controls treated with the vehicle of nitrendipine; NTR, nitrendipine (40 mg/kg); ECS + FLU, electroconvulsive shock + flunarizine; ECS + NTR, electroconvulsive shock + nitrendipine; (○), statistical significance vs. SECS group ($p < 0.01$); (■), statistical significance vs. ECS group ($p < 0.01$).

Apparatus and Procedures

Punishment-reinforced avoidance (step-down) was used as described elsewhere (17). Each rat was placed on a platform (15 × 10 cm) fixed in the center of a grid floor (50 × 30 cm). The normal exploratory reaction of stepping down onto the grid was punished by brief electric shock (50 Hz, 0.4–0.6 mA, 10 s). When the rat was placed on the platform again, its normal reaction was inhibited. Prolongation of the step-down latency was taken as a measure of retention performance. Six successive training sessions were carried out until reaching the learning criterion (remaining on the platform for at least 60 s). Retention tests were given 3 h, 24 h, and 7 days after training. Under the given experimental conditions, the test has proved sensitive to the effects of compounds found to be memory enhancing in humans (34). Behavioral analysis has demonstrated that the increase in retest latency in this paradigm reflects the learned acquisition of a specific association and is not a nonspecific effect (46). Immediately after the first correct response, electroconvulsive shock or clonidine was applied to provoke memory deficit.

Electroconvulsive shock stimulation (monophase rectangular pulses with intensity of 50 mA, single phase duration of 1 ms, stimulation frequency of 50 Hz, and trial duration of 0.5 s) by silver corneal electrodes inducing clonic seizures (30) was applied immediately after the first correct response to impair memory. A sham electroshock (SECS) was applied to the controls.

Clonidine (0.1 mg/kg) was injected IP immediately after reaching the learning criterion.

Flunarizine (10 mg/kg) and nitrendipine (40 mg/kg) were administered orally for 12 days (5 days prior to step-down training and 7 days afterward). On the fifth day, nitrendipine and flunarizine were applied 1 h before starting step-down training.

Drugs

Clonidine (Boehringer) was dissolved in distilled water and injected IP in a volume of 1 ml/kg. Nitrendipine (Chemical and Pharmaceutical Research Institute, Sofia, Bulgaria) was dissolved in distilled water to which two or three drops of Tween 80 were added. Flunarizine (Chemical and Pharmaceutical Research Institute) was dissolved in a solution of 0.1 M tartaric acid and dimethylamine acetate (17:3 v/v). Controls were treated in the same manner with distilled water or the respective vehicle in an adequate volume (1 ml/kg). The doses were chosen according to literature data and the dose–response curve determined in preliminary experiments.

Statistical Analysis

In measuring the retest latencies, a cutoff time of 60 s was chosen so that the baseline and retest experiments could be carried out at the same time of day. The percentage of rats remaining on the platform for at least 60 s was recorded. Data were assessed for significance of differences by the nonparametric Fisher's exact probability test (two-tailed) (11).

RESULTS

The animals subjected to ECS showed impaired retention, i.e., shortened latencies upon the three retention tests, in comparison to the controls with sham ECS, which suggests a pronounced retrograde amnesia. The percentage of rats reaching the learning criterion upon retention testing at 3 h, 24 h, and 7 days after training was significantly lower ($p < 0.01$) in the ECS group than in the SECS controls (Fig. 1). Both calcium antagonists tested completely abolished the ECS-induced amnesia. In the rats treated with ECS + flunarizine or ECS + nitrendipine, the percentage of animals reaching the learning

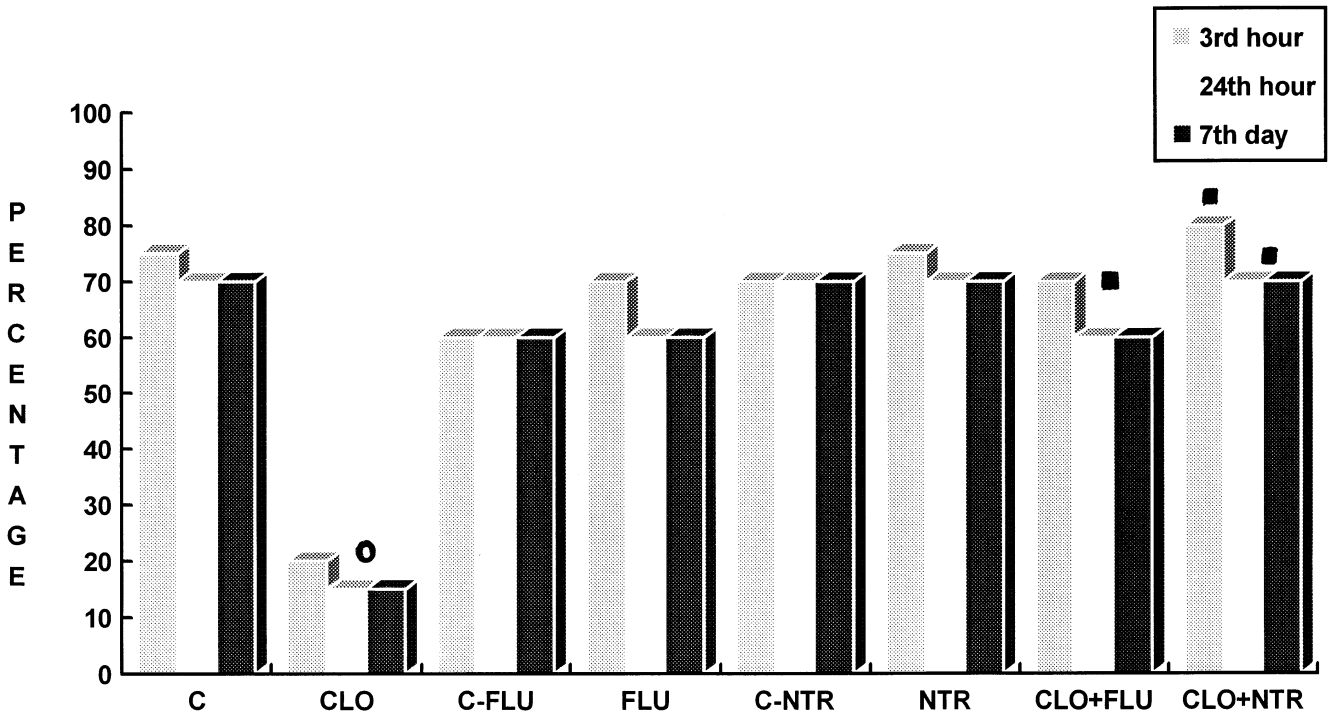

FIG. 2. Effects of flunarizine and nitrendipine on clonidine-induced memory disturbances in step-down passive avoidance in rats ($n = 10$). On the ordinate: percentage of rats attaining the learning criterion; on the abscissa: C, controls with distilled water; CL, clonidine (0.1 mg/kg); C-FLU, controls treated with the vehicle of flunarizine; FLU, flunarizine (10 mg/kg); C-NTR, controls treated with the vehicle of nitrendipine; NTR, nitrendipine (40 mg/kg); CLO + FLU, clonidine + flunarizine; CLO + NTR, clonidine + nitrendipine; (○), statistical significance vs. control group ($p < 0.01$); (■), statistical significance vs. CL group ($p < 0.01$).

criterion upon the three retention tests was considerably higher as compared with the ECS group ($p < 0.01$) and did not differ from the percentage in the SECS-treated rats (Fig. 1).

Post-training injection of clonidine also caused retrograde amnesia. The percentage of rats that had acquired the task upon retention tests given 3 h, 24 h, and 7 days after training was significantly decreased in clonidine-treated rats as compared with the vehicle-treated controls ($p < 0.01$) (Fig. 2). Nitrendipine and flunarizine antagonized the memory-impairing effect of clonidine upon the three retention tests. The percentage of rats reaching the learning criterion in the groups treated with clonidine + flunarizine and clonidine + nitrendipine was significantly higher as compared with the clonidine-treated group ($p < 0.01$) (Fig. 2).

DISCUSSION

The present study showed that the calcium channel blockers nitrendipine and flunarizine completely abolished the amnesia induced by electroconvulsive shock or by the α_2-adrenoceptor agonist clonidine in the step-down avoidance situation. This finding supports data on the retention-improving effect of calcium antagonists, especially in the case of impaired cognitive functions. It has been reported that the calcium channel blockers nicardipine, felodipine, and nifedipine have no effect on inhibitory avoidance behavior in untreated animals but significantly improve retention in hypoxic rats (48). Nimodipine improves acquisition and facilitates retention predominantly in animals with age-related cognitive disturbances (11,23,24,37). The newly synthesized piperazine derivative dotarizine, with Ca^{2+} antagonistic activity, completely prevented ECS- and pentylenetetrazole-provoked amnesia in an inhibitory avoidance situation (31).

ECS is widely used to produce retrograde amnesia through impairing consolidation and retrieval of memory traces (39). ECS induces changes in β- and α_2-adrenoceptor binding [for review, see (18)], increases the 5-HT_2-receptor number (20), and stimulates the release of brain enkephalins, causing a naloxone-reversible retrograde amnesia (25). Calcium antagonists possess anticonvulsant activity (9,47). However, this does not explain the antiamnestic effect of nitrendipine and flunarizine, as earlier data of ours have shown that such an antiamnestic effect occurs even when these drugs are applied immediately after ECS (unpublished results).

The amnestic effect of posttraining clonidine confirmed our earlier results (16,29) and those of others showing that clonidine at a low dose provokes memory disturbances in shuttle-box (21,22,27) and in water-maze trained rats (35). The α_2-adrenoceptor agonist guanfazine also has an amnestic effect that is completely antagonized by the selective α_2-adrenoceptor antagonist atipazole (38). An interesting clinical report shows deteriorated associative learning and retention in hypertensive patients continouosly treated with clonidine (13). There are but few data on the memory-improving effect of higher doses clonidine and guanfazine in old monkeys (3,4).

Both ECS (post-ictally) and clonidine provoke the occurrence of slow-wave activity, which may contribute to the amnestic effect of these agents (12). Such activity in the neocortex impairs memory consolidation in humans (28).

The exact mechanisms through which Ca^{2+} antagonists influence learning and memory are still unknown. Calcium is

essential for both seizure discharge and neurotransmitter signal transduction. The cellular correlates of learning and memory are critically dependent on the messenger role of intracellular Ca^{2+} (2,44), and it is very likely that the inhibition of Ca^{2+} entry by nitrendipine and flunarizine antagonizes the memory deficit produced by ECS or clonidine. On the other hand, calcium antagonists might influence cognitive functions via interactions with different neurotransmitter receptors and uptake processes [(8,41) etc.]. Bartfai and Vizi (6) observed that nimodipine exerts a presynaptic α_2-blocking effect, acting on calcium entry. This could explain the promnestic effect of flunarizine and nitrendipine in the case of clonidine-induced memory impairment. The role of noradrenergic–serotonergic interactions in clonidine- or ECS-provoked cognitive deficit should also be considered. Clonidine at doses of 0.1–1 mg/kg increases the firing of 5-HT cells in the dorsal raphe nucleus, facilitating the adrenergic influence on the 5-HT neurons (5,15,43). Based on these results, we suggest that not only clonidine-inhibited noradrenergic neurotransmission but also clonidine-stimulated 5-HT-ergic neurotransmission underlies the amnestic effect of the drug. In both types of amnesia, the balance between neurotransmitter systems is disturbed and there is a predominance of inhibitory influences on learning and memory (25). Bearing in mind data on the 5-HT_2-antagonistic activity of flunarizine and of some other calcium antagonists (8), the possibility that flunarizine and nitrendipine also exert their promnestic effect through inhibition of 5-HT-ergic activity should not be excluded. Such a possibility is consistent with our earlier data (17) showing that the selective 5-HT_2-antagonist ketanserine prevents ECS- or clonidine-induced amnesia in rats. Finally, the tissue distribution of these calcium antagonists and their action on cerebral circulation, metabolism, and neuronal hyperexcitability could contribute to the antiamnestic effects of these drugs (37).

In conclusion, the calcium channel blockers nitrendipine and flunarizine have a promnestic effect against experimentally induced memory deficit. This makes these drugs interesting candidates for the treatment of behavioral disturbances accompanying aging and dementia.

ACKNOWLEDGEMENT

This study was supported by grant L-316 from the National Fund (Scientific Research), Bulgaria.

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