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Nimodipine on Shuttle-box Avoidance Learning in Mice: No Impairment But Slight Improvement

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VETULANI, J., M. BATTAGLIA AND M. SANSONE. *Nimodipine on shuttle-box avoidance learning in mice: No impairment but slight improvement.* PHARMACOL BIOCHEM BEHAV **56**(4) 577–581, 1997.—The dihydropyridine calcium channel antagonist nimodipine was tested in mice of CD-1, C57BL/6, and DBA/2 strains subjected to shuttle-box avoidance training. In contrast with some findings of other authors showing impairment of shuttle-box avoidance learning by low doses of the drug in rats, nimodipine given IP before each training session at doses of 0.25, 0.5, 1, 2.5, or 5 mg/kg never impaired avoidance acquisition in mice. On the contrary, one dose of nimodipine (1 mg/kg) significantly improved avoidance acquisition in mice of the DBA/2 strain. The drug failed to impair avoidance performance in DBA/2 mice even if given acutely in the middle (third session) or at the end (fifth session) of the training period. The results contradict studies showing cognitive impairment induced by calcium channel blockers, and may provide some limited evidence in support of improved cognitive function in normal animals, although this effect is much less evident than in aged or brain-damaged subjects. © 1997 Elsevier Science Inc.

Nimodipine Avoidance learning Mice

BESIDES the well-known cardiovascular effects, calcium channel blockers exert several neuropharmacological and behavioral actions, including alteration of cognitive functions (13). The dihydropyridine derivative nimodipine has been proposed for the treatment of various central nervous system disorders (23), and preclinical studies indicated that it is able to improve learning and memory of old or brain-damaged animals (6,8,15). Learning facilitation by nimodipine has also been observed in young, neurologically intact rats (7,9,11). In contrast to this, Nikolaev and Kaczmarek (12) have reported recently that low doses of nimodipine (0.05 and 0.5 mg/kg) impaired learning in young adult Wistar rats subjected to two-way active avoidance training. Disruptive action of nimodipine on active avoidance behavior seems incongruent with our previous findings (25) showing no learning impairment in mice subjected to shuttle-box training after the administration of much higher doses of another dihydropyridine calcium channel antagonist, nifedipine. Although nifedipine was reported to penetrate the blood–brain barrier less than nimodipine does (24), at the doses used it effectively occupies the cerebral calcium channels (1).

In view of the wide clinical use of calcium channel antagonists, it seems important to assess their effects on cognitive functions in various preclinical experimental models to avoid misleading generalizations. Because of that, in the present study we tested the effects of nimodipine on shuttle-box avoidance acquisition in mice belonging to three strains—CD-1, C57BL/6, and DBA/2—that differ among themselves in learning ability in this task (2,26).

EXPERIMENT 1

The purpose of the experiment was to investigate whether daily treatment with nimodipine, in a wide range of doses, affects the acquisition of avoidance behavior. The experiment was carried out with mice of the three strains; nimodipine was injected daily, before each avoidance session, during the whole 5-day training period.

Methods

Subjects. The subjects were naive male mice, 8–9 weeks old, belonging to the randomly bred CD-1 strain and to the

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inbred C57BL/6 and DBA/2 strains (Charles River, Calco-Como, Italy). Upon their arrival in the laboratory (at least 1 week before the experiment), the mice were housed in standard transparent plastic cages (eight per cage) under standard animal room conditions (free access to food and water, 12 L:12 D cycle, ambient temperature of 23°C). The experiments were carried out between 0900 and 1600 h.

Apparatus. The apparatus consisted of eight automated shuttle-boxes, each one divided into two 20 × 10-cm compartments, connected by a 3 × 3-cm opening. A light (10 W) was switched on alternately in the two compartments and used as a conditioned stimulus (CS). The CS preceded the onset of the unconditioned stimulus (US) by 5 s and overlapped it for 25 s. The US was an electric shock (0.2 mA) applied continuously to the grid floor. The intertrial interval was 30 s. An avoidance response was recorded when the animal avoided the US by running into the dark compartment within 5 s after the onset of the CS. If animals failed to avoid the shock they could escape it by crossing during the US. Spontaneous crossings from the dark to the light compartment were punished and recorded as intertrial responses.

Drug treatment and procedure. Nimodipine (Drug Institute, Warsaw), dissolved in 50% polyethylene glycol, molecular weight 400 (PEG; Sigma), was injected intraperitoneally in a volume of 4 ml/kg; control animals received 50% PEG.

Training consisted of five daily 100-trial sessions. Mice received nimodipine, 0.25, 0.5, 1, 2.5, or 5 mg/kg, or its solvent, 30 min before each daily avoidance session. Each experimental group included eight animals.

Statistical analysis. Shuttle-box avoidance responses were evaluated by a two-factor analysis of variance (ANOVA) for each mouse strain, the factors being nimodipine (between-subject factor; six levels) and daily session (within-subject factor; five levels). Post hoc analysis was carried out with Duncan's multiple-range test.

Results

Mice of the three strains learned to escape very rapidly, and virtually no escape failure was observed during training. Intertrial responses, which were punished by electric shock, gradually disappeared as training proceeded.

Figure 1 reports, for all the experimental groups, the mean percent avoidance responses in each daily session and in the five sessions combined. Control CD-1 and C57BL/6 mice, who received PEG only, displayed rather poor performance, showing only 24–25% avoidance responses at the end of training (fifth session). Conversely, control DBA/2 mice reached high avoidance levels from the second daily session. Nimodipine had no significant effect in mice of CD-1 and C57BL/6 strains, even if some slight performance improvements were observed. Two-factor ANOVAs of the avoidance responses exhibited by mice of these two strains revealed significant main effects for training [$F(4, 168) = 37.41$ and 46.12, respectively, $p < 0.001$] but not for treatment [$F(5, 42) = 1.38$ and 0.37, $p > 0.05$], and no significant treatment × sessions interaction [$F(42, 168) = 1.05$ and 1.39, $p > 0.05$]. A two-factor ANOVA for avoidance responses of DBA/2 mice showed significant main effects of nimodipine [$F(5, 42) = 5.00$, $p < 0.01$] and training [$F(4, 168) = 538.67$, $p < 0.001$] and a significant treatment × sessions interaction [$F(20, 168) = 2.39, p < 0.01$]. A post hoc analysis indicated that various doses of nimodipine (0.5–5 mg/kg) increased avoidance resonses in the fifth daily session, but only one dose of the drug (1 mg/kg) produced a significant avoidance improvement in the whole of the five sessions combined.

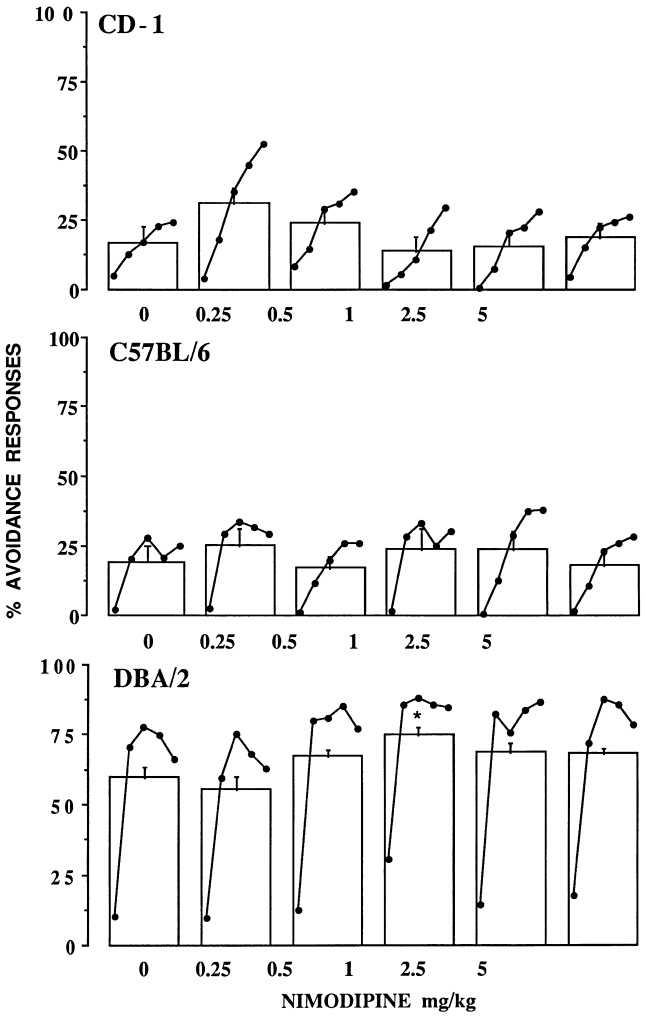

FIG. 1. Effect of nimodipine on shuttle-box avoidance acquisition in mice of CD-1, C57BL/6, and DBA/2 strains. Mean percent avoidance responses in the whole of the five 100-trial daily sessions (columns) and in each session (graphs within columns), in groups of eight mice, are shown. Vertical lines indicate SEM. Nimodipine, at doses of 0, 0.25, 0.5, 1, 2.5, or 5 mg/kg, was injected IP 30 min before each daily session. Control mice (dose 0 of nimodipine) received injections of vehicle (PEG). The asterisk indicates a significant difference, in DBA/2 mice, between nimodipine at 1 mg/kg and vehicle (Duncan's test).

EXPERIMENT 2

This experiment was designed to assess the effect of nimodipine administered acutely during the shuttle-box training and was carried out on well-performing DBA/2 mice. The study was prompted by a recent report that acute, but not subchronic, nimodipine impairs performance of mice in learning tasks (10). We presumed that an avoidance-disrupting action of acute nimodipine might be more evident if avoidance performance has not reached stable high levels. To obtain such conditions, training sessions were reduced to 50 trials each in order to lower the rate of avoidance acquisition, which was

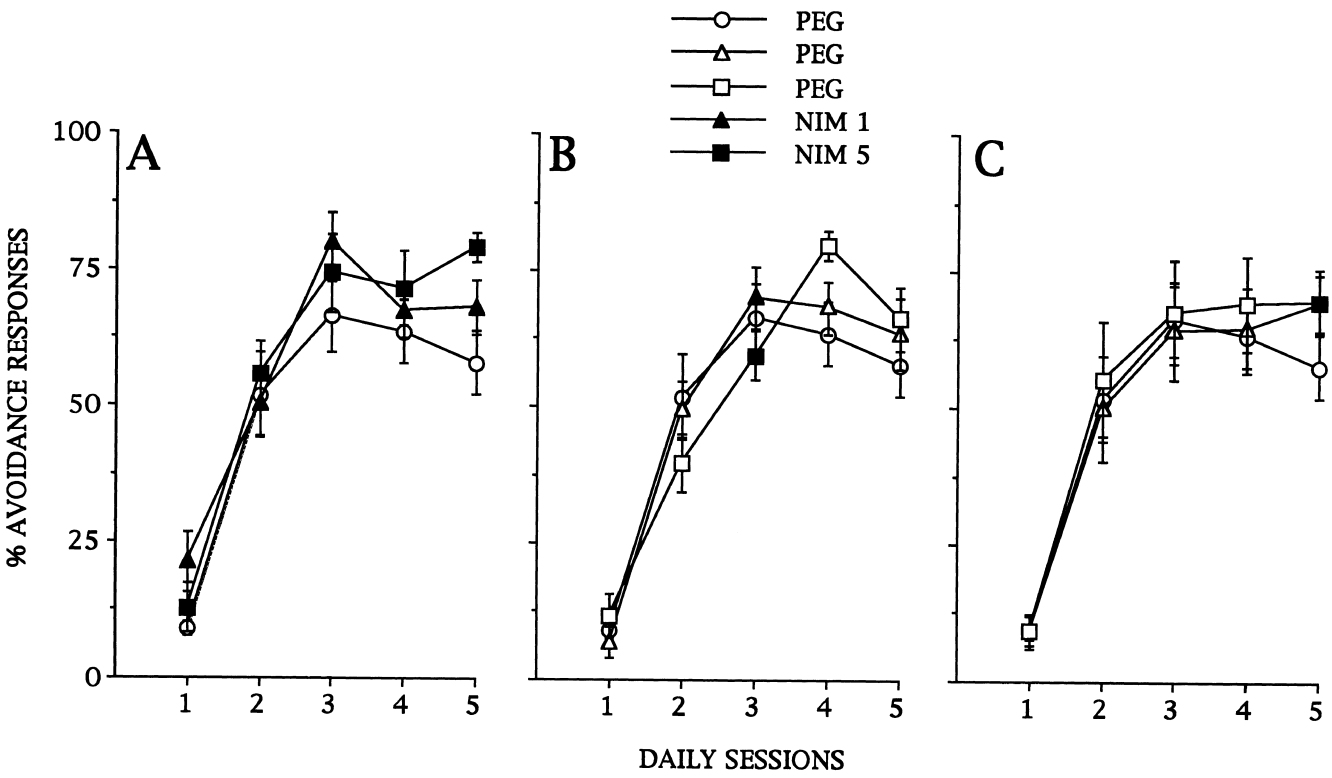

FIG. 2. Effect of nimodipine administered during the whole training period (A) or only once in the middle (third session; B) or at the end (fifth session; C) of the training, in DBA/2 mice. Mean percent avoidance responses in each of the five 50-trial daily sessions are shown. Vertical lines indicate SEM. Mice received IP injection of PEG or nimodipine, 1 or 5 mg/kg, 30 min before training. The same control group (PEG in all five sessions) is presented in all three panels.

very high, when DBA/2 mice were trained with sessions consisting of 100 trials (as was done in Experiment 1).

Methods

The subjects were naive male mice of the DBA/2 strain. The apparatus was the same as that described in Experiment 1. Nimodipine, at doses of 1 or 5 mg/kg IP, was given 30 min before each daily avoidance session or only once, before the third or the fifth session. Control injections consisted of the administration of 50% PEG. Each experimental group included eight animals. Statistical analysis was performed by a one-way ANOVA (seven levels) for each daily session.

Results

Figure 2 reports the mean percent avoidance responses for each daily shuttle-box session and each treatment group. ANOVA showed no difference among groups in any single avoidance session. Thus, avoidance performance of mice receiving nimodipine (1 or 5 mg/kg) acutely, in the third or in the fifth session, was not statistically different from that of the control group or from that of the groups treated with the corresponding dose during the whole training period.

DISCUSSION

In contrast with previous findings (12), the present results show no impairment, but slight improvement, of shuttle-box avoidance learning induced by nimodipine in mice. The use of an active avoidance test for assessing drug effects on cognitive functions may be questioned because peripheral mechanisms may be involved in avoidance performance, as, for example, in the case of the increase of active avoidance responses induced by scopolamine (22). However, improving effects of putative cognition enhancers on active avoidance learning have been reported (22) and, in particular, facilitation of shuttle-box avoidance acquisition by piracetam-like compounds has been observed in rats (27) and mice (19).

The present findings are in agreement with the prevailing view that calcium channel blocking agents may improve rather than deteriorate cognitive functions. In young adult mice of three strains, nimodipine, in a wide range of doses, never impaired shuttle-box avoidance learning either when given before each training session or when given in a single dose during the course of training. In fact, the final responses in CD-1 mice were always higher in nimodipine-treated than in control mice, although the difference did not reach the level of significance. In the high-performing DBA/2 mice, a significant avoidance improvement was produced by daily treatment with 1 mg/kg of the drug. No disruptive action of single doses of nimodipine given during training was observed.

It may be surprising that nimodipine was able to increase the number of avoidance responses in well-performing more than in poor-performing mice. It must be noted that also the nootropic drugs oxiracetam and piracetam improved shuttle-box avoidance learning in well-performing BALB/c (19) and DBA/2 (20) mice, but not in poor-performing C57BL/6 mice (17). Nevertheless, avoidance performance of C57BL/6 and

CD-1 mice was clearly enhanced when nootropics were combined with drugs exerting an alerting action, such as methamphetamine (17) and nicotine (21).

Failure of nimodipine to impair avoidance performance of mice, when given acutely during shuttle-box training, is not in agreement with findings showing impairing effects of acute administration of the drug on performance of mice in other tests, such as the Y-maze, passive avoidance, and the water maze (10). This discrepancy indicates that shuttle-box avoidance acquisition may be less sensitive to the depressant action of nimodipine than learning performance in other tests. In the present study, even 5 mg/kg of nimodipine did not impair avoidance performance, although Maurice et al. (10) found in other learning tests that even lower doses had impairing effects. Those authors suggested that this impairment might be due to interference from peripheral effects of nimodipine, leading to decrease in activity and sensitivity of animals. However, in our experience, we have frequently observed that drugs producing general behavioral depression do not impair avoidance behavior in mice. For example, the benzodiazepine tranquilizer chlordiazepoxide (16) and the serotoninomimetic agent 3-chlorophenylpiperazine (26) did not affect or even improved shuttle-box avoidance performance in mice at doses that reduce locomotor activity.

The contrast between the present findings, showing no avoidance impairment by high doses of nimodipine in mice, and the results of Nikolaev and Kaczmarek (12), indicating disruption of two-way active avoidance behavior by very low doses of the drug in rats, is more difficult to explain. It must be noted that Nikolaev and Kaczmarek (12) ascribed the avoidance-disrupting action of nimodipine in rats to impairing effects on performance rather than effects on learning and suggested that these effects could be explained by peripheral effects of the drug influencing behavior. If so, it can be supposed that peripheral side effects play a role in the behavioral action of nimodipine in rats more than in mice. It must also be noted that Nikolaev and Kaczmarek (12) used Wistar rats from their own colony. The present results show that there are some differences between strains in the responsiveness to nimodipine, and although no signs of impairment were noted in the strains of mice that we tested, it is possible that a particular substrain may respond paradoxically to nimodipine.

Moreover, some methodological points in the paper of Nikolaev and Kaczmarek (12) are unclear. The original manufacturer of nimodipine is not specified and, if the methodological details are reported correctly in their paper, it seems that they managed to maintain the substance dissolved at a concentration of 4 mg/ml in 0.2% ethanol solution.

Our results are more in agreement with findings showing facilitation of learning in intact animals (4,5,7,9,14) than with findings of cognitive impairment following the administration of calcium channel blockers. However, our data do not provide strong evidence for cognition-enhancing effects of nimodipine in normal animals, since significant effects were observed only in DBA/2 mice and only at one dose. It may be that learning facilitation by dihydropyridine calcium channel blockers is substantial only in brain-damaged (6,8,15) animals. Improvement of learning and memory may be due to a direct effect of the drugs on neuronal calcium channels (14), but the possibility cannot be excluded that calcium channel antagonists may improve learning and memory in normal subjects through an enhancement of cerebral blood flow (3). We have suggested also that potentiation by nifedipine of the facilitatory actions of amphetamine (25) and nicotine (18) on shuttle-box performance of mice may be related to the action of the calcium channel blocker on cerebral circulation.

In conclusion, our results on avoidance learning confirm the view that, even in normal animals, dihydropyridine calcium channel blockers may improve cognitive functions, although any cognition-enhancing effect is much less evident than in aged or brain-damaged subjects. The sporadic reports on disrupting effects of these compounds on avoidance learning may be due to a particular coincidence rather than reflect a general rule.

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