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Effects of combined chronic nimodipine and acute metrifonate treatment on spatial and avoidance behavior

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Abstract

The present experiment was designed to elucidate whether chronic dietary treatment with nimodipine (3 months, 1000 ppm) enhances water maze spatial navigation, passive avoidance behavior and locomotor activity, and whether such a treatment with nimodipine would interact with the therapeutic effect of acute metrifonate treatment. In young medial septum-lesioned rats, nimodipine had no effect by its own on cognitive or motor behavior, and did not enhance the water maze and passive avoidance behavior improving action of metrifonate (3 and 10 mg/kg, p.o.). Nimodipine treatment of aged rats did not markedly affect the deficit in motor performance. Single and combined nimodipine and metrifonate (3 and 10 mg/kg, p.o.) treatment of aged rats resulted in shorter escape distance values to the hidden water maze escape platform compared to those of control aged rats. The passive avoidance performance of aged rats was more effectively facilitated by a combined nimodipine and metrifonate treatment than by either of the drugs on their own. Following a washout period of 2.5 months the rats that were treated previously with nimodipine no longer performed better than aged controls in the water maze test. Furthermore, after the washout period metrifonate 10 mg/kg was no longer effective in improving the water maze behavior of the now 26-month-old rats irrespective of their chronic pretreatment. Taken together, these findings indicate that chronic nimodipine and acute metrifonate treatment may more effectively stimulate cognitive functioning than either of the treatments on their own.

Keywords: Nimodipine, chronic; Metrifonate, acute; Water maze; Passive avoidance; Motor test; Washout; Aged rat; Medial septum-lesioned rat

1. Introduction

Studies conducted using brain samples obtained from patients pathologically verified as having Alzheimer's disease have shown a consistent and marked loss of cholinergic cells in the nucleus basalis and medial septum (Whitehouse et al., 1982; Bowen et al., 1983; Bartus et al., 1985). The importance of the cholinergic neuron loss for the cognitive decline is suggested by clinical and experimental data (Decker, 1987; Hagan and Morris, 1988; Dunnett et al., 1991; Eagger and Harvey, 1995; Riekkinen et al., 1991; Soininen et al., 1992). For example, tacrine, a cholinesterase inhibitor, to some extent alleviates the clinical dementia of Alzheimer's disease patients (Knapp et al., 1992; Eagger and Harvey, 1995). However, the treatment response is modest at best and the severe dose-dependent side-effects limit the clinical use of the drug (Watkins et al., 1994). Therefore, the development of effective, better-tolerated cholinesterase inhibitors to treat the symptoms of Alzheimer's disease would be a major advance. Several drug candidates with such an improved profile have been suggested. Among these is metrifonate, a prodrug cholinesterase inhibitor which is well tolerated even after high levels of cholinesterase inhibition (Becker and Giacobini, 1988; Hinz et al., 1996) and which has been shown to improve cognitive performance in various animal models (Decker, 1987; Hagan and Morris, 1988; Riekkinen Jr. et al., 1991a,b,c,d,e, 1993) of learning and memory (Blokland et al., 1995; Van der Staay et al., 1996; Riekkinen Jr. et al., 1996; Kronforst Collins et al., 1996; Jäkälä et al., 1996). Treatment with metrifonate also enhanced cortical EEG arousal in young scopolamine-treated rats and in aged rats (Björklund et al., 1996). Moreover, if novel drugs have mechanisms of action different from those of cholinesterase inhibitors, it is theoretically possible that these drugs would be useful for add-on polytherapy with cholinesterase inhibitors to increase clinical efficacy. A

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combination of drugs may have synergistic effects in terms of efficacy and infra-additive effects on each other in terms of toxicity; i.e., an enhanced safety/efficacy ratio.

It has been hypothesized that a generalized loss of calcium homeostasis and an increase in neuronal calcium concentration occur in aging and contribute to the degeneration of neurons (Khachaturian, 1984; Peterson and Goldman, 1986; Yamada et al., 1996). According to this concept, a possible strategy to retard the effect of aging on brain structure and functioning is to decrease the intraneuronal free calcium concentration with the help of centrally active blockers of neuronal Ca^{2+} channels, such as nimodipine, a 1,4-dihydropyridine. Nimodipine selectively blocks Ca^{2+} entry through an L-type channel (McCarthy and TanPiengco, 1993) and has neuronal and cerebrovascular effects at doses producing only minor effects on the peripheral circulation. Interestingly, several studies suggest that nimodipine may provide neuroprotection following ischemic or mechanical damage to neurons (LeVere et al., 1989; Auer, 1993; Finger, 1993). Furthermore, the studies that have examined the effects of chronic nimodipine treatment have reported a treatment-induced alleviation of age-related cognitive defects (Schuurman and Traber, 1989; Fanelli, 1993).

The present study was designed to investigate the hypothesis that chronic nimodipine treatment may alleviate the age-related impairment of cognitive functioning and enhance the therapeutic effects of metrifonate. Therefore, we investigated the effects of chronic nimodipine or placebo diet on spatial navigation, passive avoidance, and motor behavior (inclined plate, free swim trial + water maze training trial swim speed, open field activity) in aged rats. Furthermore, to assess whether the therapeutic effect of an anti-cholinesterase is increased by nimodipine treatment, we compared the effects of acute oral metrifonate at subthreshold (3 mg/kg) and threshold (10 mg/kg) doses alone or in combination with chronic nimodipine treatment. The doses were selected according to our previous experience with metrifonate in these animal models (Riekkinen Jr. et al., 1996; Van der Staay et al., 1996). Finally, to evaluate further the hypothesis that chronic nimodipine treatment improves cognitive behavior in aged rats by retarding the effect of aging, we studied the effects of chronic treatment with a nimodipine-containing diet on the water maze and passive avoidance defects induced by lesions of the medial septum in young rats and on the efficacy of metrifonate to enhance the behavior of medial septum-lesioned rats.

2. Materials and methods

2.1. Rats

Forty-two young (1-month-old at the beginning of the study) male Han:Wistar rats were used in the study investigating the effects of acute metrifonate and chronic nimodipine treatments on water maze and passive avoidance performance of medial septum-lesioned rats. The sham-lesioned ($n = 6$) and half of the medial septum-lesioned ($n = 18$) rats were treated with the control diet. The other half of the medial septum-lesioned rats were fed on the nimodipine diet. The dietary treatment was continued for a period of 16 weeks. The brain lesions were made after 10 weeks of control or nimodipine treatment. Behavioral testing started 12 weeks after the initiation of the study treatments. These rats were decapitated after 16 weeks of treatment with nimodipine or control diet.

Aged (19-month-old at the beginning of the study; $n = 90$; control diet $n = 54$; nimodipine diet $n = 36$) and young (3-month-old at the beginning of the study; $n = 10$; control diet) male Han:Wistar rats were used in another experiment. In this case, the nimodipine diet and the control diet were given for 15 weeks.

The following treatment conditions were used for behavioral studies conducted with aged rats starting after 12 weeks of treatment:

free swimming and inclined plate:

- young rats: control diet;
- aged rats: control or nimodipine diet.

water maze and passive avoidance:

- young rats: control diet + metrifonate vehicle p.o. during testing days;
- aged rats: control diet + metrifonate (3 or 10 mg/kg) or metrifonate vehicle p.o. during the testing days;
- aged rats: nimodipine diet + metrifonate (3 or 10 mg/kg) or metrifonate vehicle p.o. during the testing days.

At completion of the behavioral studies, the control and the nimodipine diets had been given for 15 weeks. At this time point, all the groups of aged rats were switched to placebo diet for 2.5 months (washout period), and water maze performance and inclined plate test performance were re-assessed. The treatment conditions for inclined plate tests were the same as during the study drug feeding period (young control ($n = 8$), aged control ($n = 19$) vs. previous nimodipine ($n = 19$) diet-treated rats). In the water maze and passive avoidance studies only one dose of metrifonate was assessed in groups of previous placebo and nimodipine diet-fed aged rats:

- previous placebo diet + metrifonate vehicle ($n = 9$) or metrifonate 10 mg/kg ($n = 9$) during testing days;
- previous nimodipine diet + metrifonate placebo ($n = 9$) or metrifonate 10 mg/kg ($n = 9$) during testing days.

Young controls ($n = 8$) were also included in the re-assessment period.

2.2. Drugs

Metrifonate (a donation from Bayer/Troponwerke) was dissolved in 5% sodium citrate (pH 5.5, buffered with citric acid) and administered per os (p.o.) 30 min before

testing. For control purposes p.o. vehicle injections were used. Nimodipine-containing (1000 ppm) or control diet was continued for 3 months before the beginning of behavioral training. Food was custom adulterated with nimodipine Ssnif (Soest, Germany) to a nominal concentration of 1000 ppm, and compressed to food pellets. This corresponds to a daily dose of approximately 30 mg/kg body weight and was selected on the basis of previous pharmacological studies showing beneficial effects on various parameters of age-related neuronal degeneration (Scribane et al., 1989; De Jong et al., 1990). During all the steps of preparation and subsequent handling, the food was protected from light in order to prevent light-sensitive degradation of nimodipine.

2.3. Brain lesioning and biochemistry

Young rats were lesioned 2.5 months after the initiation of control or nimodipine diets. The rats were deeply anesthetized with chloral hydrate (350 mg/kg, i.p.) and placed in a stereotaxic frame with the bregma and lambda in the same horizontal plane. Medial septum lesions (anterior: 0.2 mm, lateral: 0.0 mm, dorsal: −7.0 mm; relative to the bregma) were produced with stainless steel electrodes (diameter 0.25 mm, 0.4 mm tip uninsulated) by passing anodal DC current (2 mA, 4 s) through the electrode. The sham-lesioned groups were treated identically, but no current was applied.

The hippocampi of sham- and medial septum-lesioned rats were dissected on ice and stored until assayed at −72°C with the radiochemical method of Fonnum (1975) for measurement of choline acetyltransferase (enzyme responsible for acetylcholine synthesis) activity. The decrease (45–55%) in choline acetyltransferase activity was similar to that which we earlier described in the study assessing the dose-response relation for metrifonate to enhance water maze navigation in medial septum-lesioned rats (Riekkinen Jr. et al., 1996).

2.4. Water maze

The circular water maze pool and computerized video-tracking system have been described in detail previously (Riekkinen Jr. et al., 1991a, 1996). The computer calculated and stored the total distance swum (in cm). The starting locations, which were labelled north, south, east and west, were located arbitrarily on the pool rim. The timing of the latency to find the submerged platform was started and ended by the experimenter. Rats were placed in the water, with their nose pointing towards the wall, at one of the starting points in a random manner.

Young medial septum-lesioned rats were tested on 4 consecutive days. Five platform trials of 70 s were assessed per day during the first 3 training days. The platform location was kept constant (in the southwest quadrant) during this period of training. On the fourth day of training the location of the escape platform was reversed to the northeast quadrant and 6 trials of 50 s were assessed. In each trial (day 1–4), the rats were allowed to stay on the platform for 10 s. If a rat failed to find the platform during the maximum duration of the trial, the experimenter placed the rat on the platform for 10 s. A 30-s recovery period was allowed between the training trials.

The water maze training schedule of aged rats was as follows. On the first day no platform was in the pool and the rats swam freely for 70 s. Then, during the next 5 consecutive days two training trials of 70 s were assessed and the submerged platform was constantly located in the southwest quadrant. After the rats found the escape platform, they were allowed to stay on it for 5 s. An intertrial interval of 30 s was allowed after the rats were removed from the platform. Those rats that did not find the platform in 70 s were placed on the platform by the tester for 5 s.

2.5. Passive avoidance

Passive avoidance training was started 24 h after the last water maze testing session. The passive avoidance box had a light and a dark compartment. During the training trial the rats were placed in the light compartment. Thirty seconds later the sliding guillotine door was opened. After the rat entered the dark compartment, the door was closed and a foot shock of 1.0 mA (3 s) was given. During the testing trial 72 h later the rat was again placed in the light compartment, and the latency to enter the dark compartment was measured (360 s maximum latency).

2.6. Inclined plate

Rats were placed in the middle of an inclined (30°) smooth rubber plate (150 × 50 cm), facing the wall. The time the rats could stay on the platform was measured (s).

2.7. Statistics

Multiple analysis of variance was used for calculating group differences in escape distance values measured during the first 3 days of training. Comparisons between the water maze data of the last testing day and passive avoidance values of different groups were made with one-way analysis of variance followed by Duncan's post-hoc multiple group comparisons. $P < 0.05$ was accepted as significant.

3. Results

3.1. Medial septum-lesioned rats

3.1.1. Water maze

During reference memory testing (day 1–3) in the water maze, a significant overall effect was observed ($F(6,35) =$

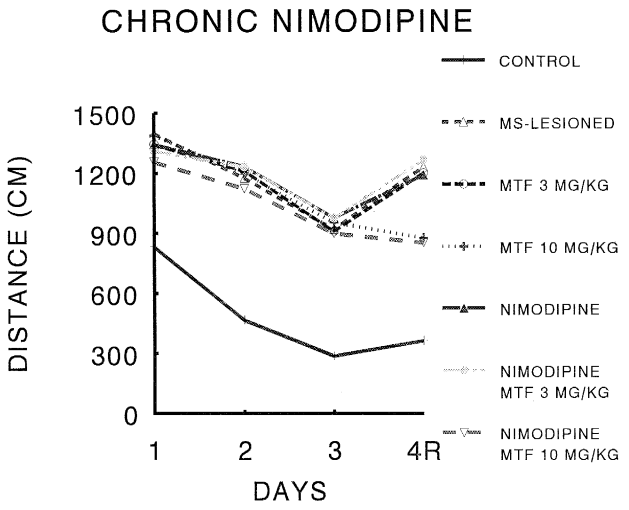


Fig. 1. Effects of chronic nimodipine treatment (3 months, 1000 ppm in the diet) on water maze spatial navigation in saline- and metrifonate- (3 and 10 mg/kg, p.o.) treated medial septum-lesioned rats. Nimodipine treatment did not modulate behavior of vehicle or metrifonate-treated medial septum-lesioned rats. *Y*-axis: escape distance in cm, group means of daily training trials. *X*-axis: training days 1–3 (fixed platform location), day 4 (platform reversal). Abbreviations: CONTROL, MS-LESIONED = medial septum-lesioned, NIMODIPINE = chronic nimodipine, MTF3/10 = metrifonate 3 or 10 mg/kg.

19.82, $P < 0.001$) (Fig. 1). Controls acquired the task more rapidly than any other groups ($F(1,10) > 30$, $P < 0.01$). Single or combined metrifonate 3 or 10 mg/kg and chronic nimodipine treatments did not improve the water maze acquisition behavior of medial septum-lesioned rats ($F(1,10) < 1$, $P > 0.1$, for all comparisons).

During the platform reversal phase, medial septum lesion induced a significant defect ($P < 0.05$) and metrifonate 10 mg/kg improved ($P < 0.05$) the spatial navigation of medial septum-lesioned rats ($F(6,35) = 14.73$, $P < 0.001$). Nimodipine did not modulate the behavior of medial septum-lesioned rats and did not change the performance-improving effect of metrifonate on water maze navigation ($P > 0.05$; for all comparisons).

The swim speed of medial septum-lesioned rats was increased ($F(6,35) = 3.0$, $P < 0.001$). The treatments used in this study did not significantly modulate the swim speed of medial septum-lesioned rats ($F(1,10) < 1$, $P > 0.05$) (sham-lesioned: 25 ± 2; medial septum-lesioned: 29 ± 2; medial septum-lesioned + nimodipine: 29 ± 2; medial septum-lesioned + nimodipine + metrifonate 3 mg/kg: 30 ± 2; medial septum-lesioned + nimodipine + metrifonate 10 mg/kg: 29 ± 2; medial septum-lesioned + metrifonate 3 mg/kg: 30 ± 1; medial septum-lesioned + metrifonate 10 mg/kg: 31 ± 2; m/s, mean $\pm$ S.D.).

3.1.2. Passive avoidance

The entry latency during the training trial did not differ between the groups ($F(6,35) = 0.21$, $P = 0.93$). In contrast, during the test trial medial septum-lesioned rats had an impaired performance ($F(6,35) = 6.4$, $P < 0.0001$) (Fig. 2). Comparison of the entry latencies revealed that none of the single or combined study drug treatments had any effect on passive avoidance latency ($P > 0.05$ vs. medial septum-lesioned control-treated rats).

3.2. Aged rats

3.2.1. Motor activity tests

In the inclined plate and free swimming tests, we compared the performance of young controls, aged controls and the aged nimodipine group.

3.2.2. Inclined plate, chronic treatment period

Aged rats had and impaired inclined plate performance ($F(2,85) = 357.9$, $P < 0.0001$) (Fig. 3a). Nimodipine treatment had a statistically significant but small ameliorating effect on the age-related decline of inclined plate performance ($P < 0.05$).

3.2.3. Inclined plate, washout period

Aged rats had an impaired inclined plate performance ($F(1,44) = 91.8$. $P < 0.0001$) (Fig. 3a). The group that was previously treated with nimodipine did not perform better than the group that was treated with a placebo diet ($P > 0.05$).

3.2.4. Free swimming, chronic treatment period

During the water maze free swimming trial, young rats swam faster than aged rats ($F(2,87) = 12.0$, $P < 0.0001$). Nimodipine did not modulate the speed of swimming of aged rats ($P > 0.05$) (Fig. 3b).

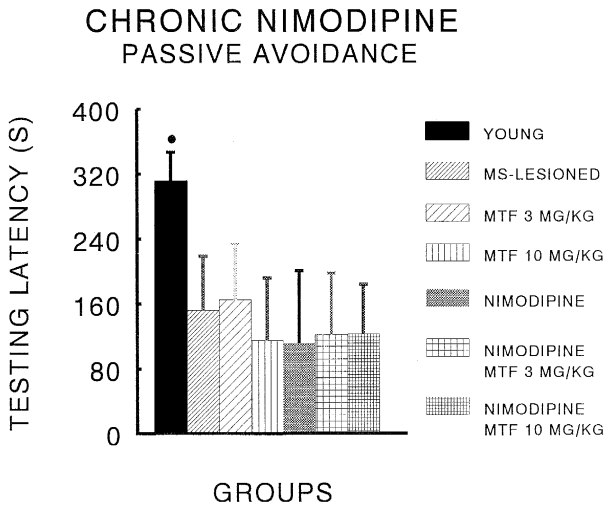
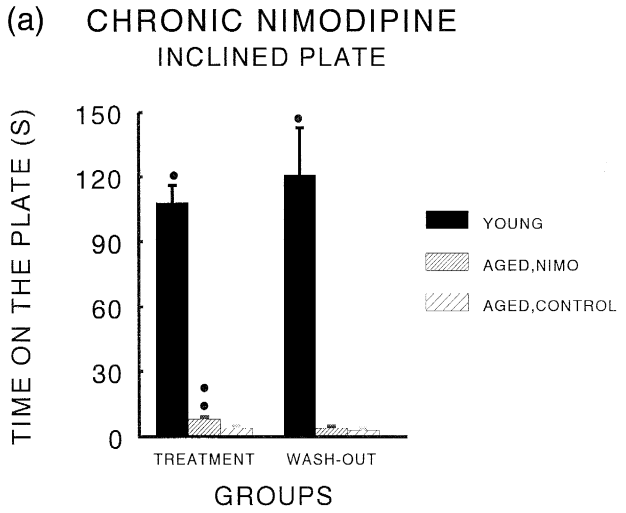


Fig. 2. Effects of chronic nimodipine (3 months, 1000 ppm, in the diet) treatment on passive avoidance in saline- and metrifonate- (3 and 10 mg/kg, p.o.) treated medial septum-lesioned rats. Nimodipine treatment did not modulate behavior of vehicle or metrifonate-treated medial septum-lesioned rats. *y*-axis: testing entry latency in seconds, group mean $\pm$ S.D. Abbreviations: CONTROL, MS-LESION = medial septum-lesioned, NIMODIPINE = chronic nimodipine, MTF3/10 = metrifonate 3 or 10 mg/kg. $^{\bullet}$ $P < 0.05$ vs. all the other groups.

3.2.5. Water maze, chronic treatment period

A significant overall effect was observed ($F(6,81) = 2.54$, $P = 0.026$) (Fig. 4a). Aged control diet-treated rats were clearly impaired compared with young rats ($F(1,22) = 14.3$, $P = 0.0005$), but nimodipine-treated aged rats were not impaired ($F(1,22) = 0.36$, $P = 0.28$). Metrifonate 3 ($F(1,22) = 1.2$, $P = 0.14$) or 10 ($F(1,22) = 2.9$, $P = 0.05$) mg/kg-treated aged rats swam a shorter distance during the training period than aged controls. Chronic nimodipine treatment alleviated the age-related increase in escape distance values ($F(1,22) = 3.34$, $P = 0.04$). The combination of nimodipine and metrifonate 3 and 10 mg/kg ($F(1,22) = 8.11$ and 4.34, respectively, $P < 0.05$) significantly decreased water maze escape distance values compared with those of control-treated aged rats.

During the acquisition of the escape task, the swim speeds of the animals were also analyzed and a marked overall group difference was observed ($F(6,81) = 4.57$, $P < 0.0001$). Metrifonate 3 mg/kg ($F(1,22) = 0.44$, $P = 0.26$) had no effect on swim speed, but the dose of 10 mg/kg ($F(1,22) = 4.6$, $P = 0.02$) decreased the swim speed of aged rats. Similarly, the higher dose of metrifonate slightly decreased the swim speed of aged rats fed on the nimodipine diet ($F(1,22) = 3.1$, $P = 0.05$), while the lower dose did not ($F(1,22) = 0.28$, $P = 0.3$). Ni-



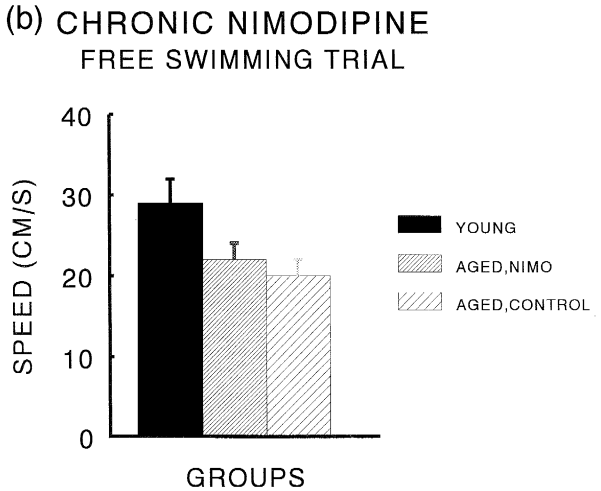
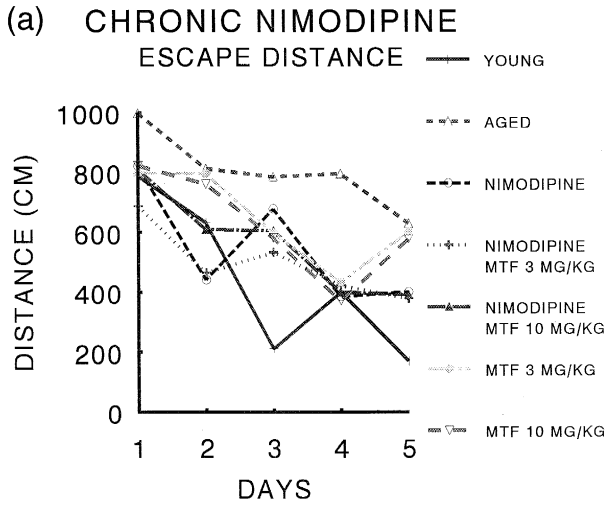


Fig. 3. Effects of chronic nimodipine (3 months, 1000 ppm, in the diet) treatment on age-related failure of motor measures: inclined plate and free swimming performance in aged rats. Part a: Inclined plate. Note a slight but statistically significant effect of nimodipine treatment on inclined plate performance at the end of the chronic treatment period. However, the groups did not differ after a washout period of 2.5 months. Abbreviation: NIMO = nimodipine. *y*-axis: latency that rats could stay on the inclined rubber plate, mean ± S.D. • $P < 0.05$ vs. all the other groups. •• $P < 0.05$ vs. aged control diet treated rats. Part b: Free swimming trial. Note a lack of an effect of nimodipine on motor performance (swimming speed) in aged rats. Aged rats were again divided into two groups only based on their diet. Abbreviation: NIMO = nimodipine. *Y*-axis: speed in cm/s, mean ± S.D.



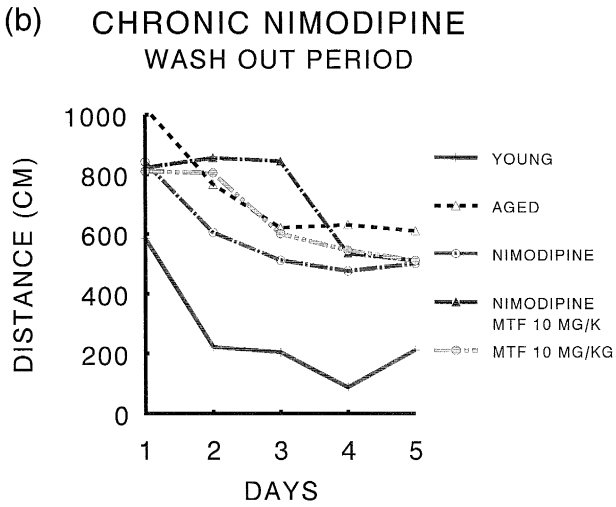


Fig. 4. (a) Effects of chronic nimodipine treatment (3 months, 1000 ppm, in the diet) on water maze spatial navigation in saline- and metrifonate- (3 and 10 mg/kg, p.o.) treated aged (23.5-month-old) rats. (b) Water maze spatial navigation performance was impaired after the 2.5-month washout period equally in all groups of aged rats, and 10 mg/kg metrifonate (p.o.) had no effect on spatial navigation failure. Abbreviation: MTF = metrifonate. *y*-axis: escape distance in cm, group means of daily training trials. *x*-axis: training days 1–5.

modipine treatment alone did not affect the swim speed of aged rats ($F(1,22) = 1.0$, $P = 0.16$) (young: 26 ± 2; aged control: 23 ± 1; aged + metrifonate 3 mg/kg: 23 ± 2; aged + metrifonate 10 mg/kg: 20 ± 1; aged + nimodipine: 23 ± 1; aged + nimodipine + metrifonate 3 mg/kg: 22 ± 2; aged + nimodipine + metrifonate 10 mg/kg: 21 ± 1; m/s, mean $\pm$ S.D).

3.2.6. Water maze, washout period

A marked overall group effect was observed, and comparison of young controls with all the different groups of aged rats revealed that aging impaired water maze navigation performance ($F(1,15) = 194$, $P < 0.0001$; for all comparisons) (Fig. 4b). None of the single or combined treatments improved the water maze performance of aged rats ($F(1,15/17) < 1$, $P > 0.1$; for all comparisons). Further, the group that had been on the nimodipine diet was not better than the aged control group ($F(1,16) = 0.8$, $P = 0.19$).

Aged rats swam slower ($F(1,15) = 19.31$, $P < 0.001$) and none of the treatments affected the swim speed of aged rats ($F(1,17/16) < 1.0$, $P > 0.3$; for all comparisons) (young: 25 ± 2; aged control: 20 ± 2; aged + metrifonate 20 ± 2; aged ex-nimodipine: 21 ± 1; aged ex-nimodipine + metrifonate 10 mg/kg: 20 ± 2; m/s, mean $\pm$ S.D.).

3.2.7. Passive avoidance, chronic treatment period

The number of shocks delivered to different groups during the training trial was nearly identical ($F(6,81) = 0.77$, $P = 0.30$) (Fig. 5). During the training trial, the first entry latency of aged controls and chronic nimodipine + metrifonate 3 mg/kg-treated rats was slightly longer than that of the young rats (overall group effect: $F(6,81) = 1.72$, $P = 0.13$; young rats vs. old chronic nimodipine + metrifonate 3 mg/kg treated: $P < 0.03$; Duncan's post-hoc test). However, no significant differences were found between the different groups of aged rats during the training session ($P > 0.05$).

During the test trial, aged rats had an impaired performance (overall effect: $F(6,81) = 9.1$, $P < 0.0001$; Duncan's post-hoc test: $P < 0.05$; young controls vs. aged controls) (Fig. 5). Comparison of the entry latencies revealed that nimodipine-treated rats (acute placebo, metrifonate 3 or 10 mg/kg) had higher group mean values than chronic placebo-treated rats. A two-way-analysis of variance (diet $\times$ drug) revealed significant diet ($F(1,71) = 6.2$, $P < 0.01$) and drug ($F(2,71) = 3.8$, $P < 0.01$) effects, but the diet $\times$ drug interaction ($F(2,71) = 0.39$, $P = 0.33$) was not significant. This statistical analysis indicates that chronic nimodipine treatment improved the performance of aged rats in the passive avoidance test. Acute metrifonate treatment also stimulated passive avoidance behavior in aged rats. While both the 3 and 10 mg/kg metrifonate doses were effective in rats pretreated with nimodipine ($P < 0.05$; aged control rats vs. nimodipine + metrifonate 3 or 10 mg/kg-treated aged rats), only the higher dose group reached the level of significance of the control diet-fed rats ($P < 0.05$; aged control rats vs. metrifonate 10 mg/kg-treated aged rats/$P > 0.05$; aged control rats vs. metrifonate 3 mg/kg treated aged rats).

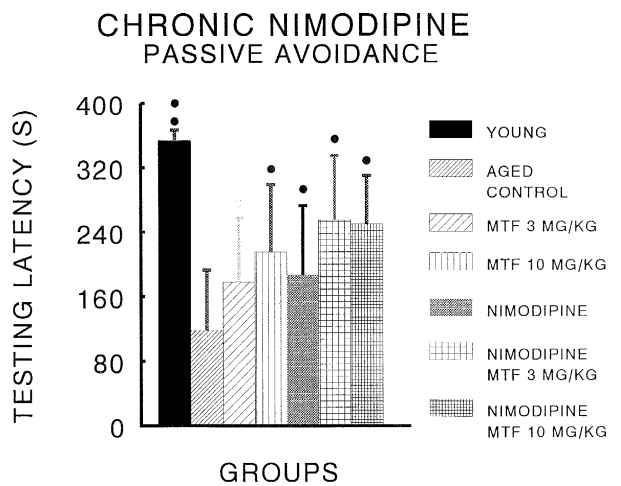


Fig. 5. Effects of chronic nimodipine (3 months, 1000 ppm) diet on passive avoidance behavior in saline- and metrifonate- (3 and 10 mg/kg, p.o.) treated aged rats. Note that nimodipine-treated rats performed better than control diet-treated aged rats. Further, metrifonate 3 mg/kg + nimodipine-treated rats performed better than aged controls, but single metrifonate 3 mg/kg treatment had no effect. Both of the groups treated with metrifonate 10 mg/kg (control or nimodipine diet) performed better than the aged controls. Abbreviation: MTF = metrifonate. *y*-axis: testing entry latency in seconds, group mean $\pm$ S.D. $^{\bullet\bullet}$ $P < 0.05$ vs. all the other groups. $^{\bullet}$ $P < 0.05$ vs. aged controls.

4. Discussion

The present results show that chronic nimodipine administered in the diet and acute treatment with metrifonate, alone and in combination, alleviated the age-related failure of water maze spatial navigation and passive avoidance behavior. In contrast, the effect of nimodipine on motor activity measures was rather modest. Our control study with young medial septum-lesioned rats showed that chronic nimodipine treatment did not facilitate water maze spatial navigation performance, enhance the therapeutic effect of metrifonate or alleviate the decrease in hippocampal choline acetyltransferase activity induced by medial septum lesioning. Further, the motor hyperactivity in young medial septum-lesioned rats was not affected by single or combined study treatments.

Our observation that nimodipine treatment for 3.5 months improved the water maze and passive avoidance performance of aged rats is supported by data showing that chronic administration of an L-type calcium channel blocker may alleviate the cognitive decline observed in aging (Schuurman and Traber, 1989; Ingram et al., 1994). Several studies have reported that even acute oral nimodipine treatment may alleviate some of the age-related cogni-

tive defects (Schuurman and Traber, 1989; Sandin et al., 1990; LeVere and Walker, 1991). Contrary to these studies, we failed to observe any beneficial effect of acute daily (4–10 days) pretraining treatment with various doses of nimodipine (3–30 mg/kg p.o.) on the water maze memory or passive avoidance defect in 26- to 28-month-old or young control, scopolamine-treated or medial septum-lesioned rats (Jäkälä et al., 1996). It cannot be excluded that the lack of an effect of acute nimodipine treatment on water maze and passive avoidance behavior in our aged rats may be due to age and strain differences of experimental animals and/or to the sensitivity of the paradigm used for detecting acute nimodipine treatment-induced behavioral effects in aged rats. Our present results further indicate that the effect of nimodipine treatment to improve water maze and passive avoidance behavior is specific for the aging rat, because chronic nimodipine treatment did not improve water maze and passive avoidance behavior, or diminish the loss of cholinergic fibers in the hippocampus of medial septum-lesioned young rats.

In contrast, the stimulating effects of metrifonate on cognitive behavior are not restricted to the aging rat. In line with previous observations from our and other laboratories, metrifonate had beneficial effects in both medial septum-lesioned and aged rats, although the profile was somewhat different. In young lesioned animals, the compound had no effect on acquisition of the platform location during the first 3 days of testing. A beneficial effect was only observed on reversal learning after changing the platform position, confirming our earlier results (Riekkinen Jr. et al., 1996; Jäkälä et al., 1996). Aged rats, however, benefit from the same dose of metrifonate (10 mg/kg p.o.), showing an enhanced acquisition (Blokland et al., 1995; Van der Staay et al., 1996; the present study) of the water maze and passive avoidance, while reversal learning in water maze is unaffected (Riekkinen Jr. et al., 1996; Jäkälä et al., 1996; the present study). Interestingly, the increase in acquisition by metrifonate is not limited to the Morris task or old rats (Van der Staay et al., 1996; Kronforst Collins et al., 1996). It should be noted that the difference between aged and medial septum-lesioned rats is not all-or-nothing. Doses of metrifonate (30–100 mg/kg p.o.) higher than those used in the present study (3–10 mg/kg p.o.) to test a possible additive or synergistic interaction with nimodipine, are well documented to improve acquisition and retention in Morris water escape and passive avoidance tests in young medial septum-lesioned rats (Riekkinen Jr. et al., 1996). Taken together, it appears that the acquisition and retention of spatial navigation or passive avoidance tasks in medial septum-lesioned rats is less sensitive to modulation by metrifonate than reversal learning, while the situation is different in aged rats. The resulting implications for the neurobiological pathways involved in cognitive processing in these different types of memory and animal models remain to be assessed.

An interesting and novel finding of our study is that in aged rats combined acute metrifonate and chronic nimodipine treatment had a greater therapeutic effect on passive avoidance behavior than either of the treatments alone. Further, the present and previous results (Jäkälä et al., 1996) demonstrated that acute or chronic p.o. nimodipine treatment neither attenuated nor enhanced the efficacy of metrifonate to stimulate water maze or passive avoidance behavior in young scopolamine-treated and medial septum-lesioned rats. Thus, chronic nimodipine and acute metrifonate treatments appear to act via independent biological mechanisms to facilitate spatial reference memory and avoidance behavior. Chronic nimodipine treatment may slow down the age-related degeneration of brain mechanisms important for water maze and passive avoidance behavior (Khachaturian, 1984; Wenk et al., 1991; Miettinen et al., 1993), and acute metrifonate treatment may, by enhancing cholinergic activity, stimulate the functioning of brain mechanisms underlying water maze and passive avoidance behavior (Blokland et al., 1995; Van der Staay et al., 1996; Riekkinen Jr. et al., 1996). The apparent mutual independence of the involved drug targets and physiological pathways opens interesting new possibilities for a more effective treatment of progressive cognitive disorders occurring later in life, such as senile dementia of the Alzheimer type.

As opposed to cognitive parameters, the age-related decline in (swim speed, inclined plate) measures of motor performance was not effectively modulated by nimodipine diet. Previous studies have reported either improvement (Schuurman and Traber, 1989) or no change (Ingram et al., 1994) in motor activity following chronic nimodipine treatment of aged rats at a dose that stimulated cognitive functioning. Thus, it is possible that different tests used to assess motor activity may reveal different effects of nimodipine, dependent, for instance, on the test difficulty.

Also the effects of metrifonate on swim speed in young medial septum-lesioned and aged rats were measured during the memory acquisition trials in this study. In young medial septum-lesioned rats metrifonate at a dose that alleviated the water maze failure had no effect on the swim speed. Metrifonate 10 mg/kg slightly slowed the swim speed of aged rats during reference memory testing. A similar effect was previously found in 27-month-old Wistar rats (Riekkinen Jr. et al., 1996). However, it should be noted that the locomotor-suppressing effect of metrifonate at behaviorally active doses (10–12.5 mg/kg) is not consistently observed in Morris water escape tests with aged rats. Thus, in our re-assessment experiment after the washout of nimodipine, it did not show up, even though the rats were 26 months old at this stage. Similarly, we (Riekkinen Jr. et al., 1996) and others (Blokland et al., 1995; Van der Staay et al., 1996) did not find a reduction in swim speed after treatment with metrifonate at 10 or 12.5 mg/kg in 19–25-month-old Wistar rats.

In conclusion, the present results show that combined chronic nimodipine and acute metrifonate treatment effec-

tively stimulates cognitive functioning in aged rats and that the combined administration of these drugs may have a better effect on performance than either of the treatments alone.

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