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Effects of combined nimodipine and metrifonate on rat cognition and cortical EEG

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Abstract

The present study investigated if short-term treatment with an L-type Ca^{2+}-channel inhibitor, nimodipine, can stimulate cognitive functioning and cortical electroencephalograph (EEG) arousal, and potentiate the effect of a cholinesterase inhibitor, metrifonate. Pretraining administration of nimodipine (3, 10 and 30 mg/kg, p.o.) had no effect on water maze and passive avoidance behavior of young neurologically intact controls, or water maze and passive avoidance performance failure induced by scopolamine pretreatment (i.p.; 0.4 mg/kg during the water maze and 2.0 mg/kg during the passive avoidance study), medial septal lesioning, or aging. Furthermore, nimodipine (3, 10 and 30 mg/kg, p.o.) had no effect on the improvement by metrifonate (10 mg/kg, p.o.) of the water maze and passive avoidance failure induced by scopolamine pretreatment or medial septal lesioning, nor did it affect the potential of metrifonate (30 mg/kg, p.o.) to improve the water maze or passive avoidance behavior of aged rats. Finally, nimodipine (3, 10 and 30 mg/kg, p.o.) had no effect on spontaneously occurring thalamically generated neocortical high-voltage spindles or spectral EEG activity of young controls, nor did it alleviate the spectral EEG abnormality induced by scopolamine (0.2 mg/kg, i.p.) administration. Also, the combination of nimodipine 3 or 10 mg/kg and a subthreshold dose of metrifonate 10 mg/kg could not suppress high-voltage spindles or scopolamine treatment-induced spectral EEG activity abnormalities. According to the present results, short-term treatment with nimodipine does not stimulate cognitive functions or increase cortical EEG arousal, and does not block or potentiate the propensity of metrifonate to improve cognitive performance of rats.

Keywords: Nimodipine; Metrifonate; Cognitive function; EEG (electroencephalograph), cortical; (Rat)

1. Introduction

The reports from clinical studies showing that the loss of cholinergic cells in the basal forebrain during Alzheimer's disease may to some extent be responsible for the decline in cognitive functioning kindled interest in the behavioral functions of the cholinergic cells (Whitehouse et al., 1982; Bowen et al., 1983; Bartus et al., 1985; Bowen and Davison, 1986; Reinikainen et al., 1990). For example, decreased choline acetyltransferase activity in post mortem frontal cortex in confirmed Alzheimer's disease patients could be correlated with a severe slowing of neocortical electroencephalogram (EEG) waves (Soininen et al., 1992) and an anti-cholinesterase, tetrahydroaminoacridine, suppressed to some extent the slow waves in patients with Alzheimer's disease and alleviated the cognitive decline associated with this disease (Eagger et al., 1991; Riekkinen et al., 1991; Knapp et al., 1992; Eagger and Harvey, 1995). However, the clinical use of tetrahydroaminoacridine is limited due to liver toxicity and rather modest therapeutic effects (Eagger et al., 1991, 1992; Watkins et al., 1994). Therefore, a need for the development of novel and effective anti-cholinesterase drugs for the treatment of Alzheimer's disease is indicated. Metrifonate is a potent and long-acting cholinesterase inhibitor that has a considerably broader therapeutic window and fewer side effects in animal cognition models than tetrahydroaminoacridine (Blokland et al., 1995; Nabeshima et al., 1995; Van der Staay et al., 1996a; Björklund et al., 1996; Riekkinen Jr. et al., 1996a).

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One pharmacological model for the study of the efficacy of novel cholinesterase inhibitors to promote the activity of damaged cholinergic pathways is to investigate if such compounds can stimulate water maze and passive avoidance behavior in young rats treated with scopolamine, a muscarinic acetylcholine receptor antagonist, and in medial septal-lesioned, or aged rats (Decker, 1987; Hagan and Morris, 1988; Riekkinen Jr. et al., 1990a,b, 1991a,c,e, 1992; Decker and McGaugh, 1991; Levine, 1992;). We have reported that metrifonate treatment stimulates water maze and passive avoidance behaviors effectively in these animal models of learning and memory over a broad dose range (Riekkinen Jr. et al., 1996a). In animal models of cholinergic deafferentation we observed that metrifonate 10–100 mg/kg (p.o.) stimulated water maze spatial navigation and at 30–100 mg/kg (p.o.) stimulated passive avoidance performance (Riekkinen Jr. et al., 1996a). Furthermore, Blokland et al. (1995) and Van der Staay et al. (1996a) described that metrifonate stimulated water maze spatial navigation in 19-month-old and 25-month-old rats in the dose range of 10–30 mg/kg (p.o.). Previously, we had reported that metrifonate at 10 and 30 mg/kg (p.o.) improved water maze spatial navigation and at 30 mg/kg (p.o.) improved passive avoidance of 23-month-old rats. However, in 27-month-old rats metrifonate failed to stimulate water maze performance, but enhanced passive avoidance behavior at 10 and 30 mg/kg (p.o.) (Riekkinen Jr. et al., 1996a). These results indicate that metrifonate effectively stimulates water maze and passive avoidance performance in some animal cognition models.

Experimental EEG studies investigating the effects of drugs on neocortical slow waves and thalamocortically generated waking-immobility-related neocortical spike-and-wave discharges (high-voltage spindles) may also be a suitable pharmacological model for testing the efficacy of novel therapies to stimulate the function of acetylcholine synapses involved in the modulation of brain electrical arousal (Riekkinen Jr. et al., 1991b,d, 1992, 1993; Björklund et al., 1996). For example, in young rats, scopolamine treatment increases slow waves in neocortical EEG polygraph recordings (Riekkinen Jr. et al., 1991b, 1993; Björklund et al., 1996). It is noteworthy that we (Björklund et al., 1996) reported that metrifonate suppressed the scopolamine treatment-induced abnormality of EEG activity more effectively than did tetrahydroaminoacridine. Furthermore, metrifonate (30–60 mg/kg) suppressed spontaneously occurring high-voltage spindles in young and in aged rats (Björklund et al., 1996). Therefore, metrifonate treatment also enhances the cholinergic modulation of cortical electrical arousal.

However, it is also important to develop non-cholinergic drug therapies for Alzheimer's disease that would increase the palliative therapeutic action of cholinergic drugs or slow down the progression of the disease. Indeed, in the treatment of other neurological disorders, such as epilepsy, rational polypharmacy can be used to decrease the dose-related side effects and increase the therapeutic effect. Another pharmacological treatment strategy that has been suggested to be effective in alleviating age-related neurodegeneration and cognitive dysfunction is to use drugs that modulate Ca^{2+} metabolism. Such drugs may have therapeutic effects in the neuroprotective treatment of patients with Alzheimer's disease (Khachaturian, 1984; Peterson and Goldman, 1986). Ca^{2+} homeostasis is impaired in Alzheimer's disease and loss of Ca^{2+} regulation may be involved in Alzheimer's disease-related neuronal degeneration. In rats, chronic treatment with nimodipine alleviates the age-related decline of memory (LeVere and Walker, 1991). Importantly, Ca^{2+}-channel inhibitors may also acutely modulate cognitive functioning (Deyo et al., 1989, 1992; LeVere et al., 1989; Deyo, 1990; Sandin et al., 1990; LeVere and Walker, 1991; Quartermain et al., 1993; Maurice et al., 1995). Nimodipine facilitates the recovery of preoperatively learned two-choice brightness discrimination following decortication of visual areas even when the drug was initially administered two weeks after the neural insult (LeVere et al., 1989). On the contrary, the reduction of Ca^{2+} entry into the neurons may retard the induction of long-term potentiation, and in young adult mice short-term nimodipine treatment markedly impairs spontaneous alternation, water maze navigation and passive avoidance behaviors (Maurice et al., 1995). Therefore, it is possible that the effect of nimodipine on cognition may depend on the cognitive test, the animal species and the model used (brain-damaged vs. normal subjects).

The first aim of the present study was to investigate the effects of short-term nimodipine treatment on water maze spatial navigation and passive avoidance performance in various rat cognition models (intact young and old rats, scopolamine-treated and medial septum-lesioned young rats) and on cortical EEG arousal. The second aim was to investigate if short-term nimodipine treatment potentiates the metrifonate treatment-induced improvement of water maze and passive avoidance performance. Short-term treatment effects of nimodipine were studied first as a basis for subsequent chronic studies. For example, in an acute treatment schedule, pharmacokinetic or toxicologic interactions could show up, indicating limits for a subsequent chronic approach. We hypothesized that additive or synergistic effects between nimodipine and metrifonate treatments could be seen, based on the reported cognition-enhancing properties of both of these compounds. On the other hand, influx of Ca^{2+} is a critical factor in the induction of long-term potentiation, a model of learning and memory that is known to be strengthened by acetylcholine (Burgard and Sarvey, 1990). Therefore, as nimodipine directly influences Ca^{2+} influx into neurons, inhibitory interactions between nimodipine and metrifonate treatments could also have occurred. Low doses of metrifonate were selected to maximize the possibility of detecting any modulatory effect of nimodipine on the behavioral effects of metrifonate.

2. Materials and methods

2.1. Animals

Young (3-month-old) and aged (26–28-month-old) male Han:Wistar rats were used in the present study. Four separate groups of rats were used: (1) young intact, (2) young medial-septal lesioned and (3) aged rats for water maze and passive avoidance studies, and (4) another group of young intact rats for EEG studies. For experimental groups and training schedule see legends of Fig. 1 and Tables 1 and 2. The rats were housed three per cage in a controlled environment (20 ± 2°C, humidity at 50–60%, light period 07:00–19:00). Food and water were ad lib. The rats to be used for EEG recordings were housed singly after implantation of EEG electrodes. The Local Ethical Committee and Provincial Government of Kuopio had given us permission to carry out this study.

2.2. Drugs

Nimodipine (3, 10 and 30 mg/kg) (a gift from Troponwerke) was suspended in 1% tylose and injected daily p.o. (5 ml/kg) 30 min before testing. Metrifonate (10 or 30 mg/kg) (a gift from Troponwerke) was dissolved in 5% sodium citrate (pH 5.5, buffered with citric acid) and injected daily p.o. 30 min before testing. Scopolamine hydrobromide (0.2, 0.4 or 2 mg/kg) (Merck) was dissolved in 0.9% NaCl and injected daily i.p. 35 min before testing. Young and aged controls received vehicle injections (p.o., p.o + p.o., p.o. + i.p. or p.o. + p.o. + i.p.) of equal volumes. The control-treated groups will be termed saline-treated rats. Scopolamine 0.2, 0.4 and 2 mg/kg doses were used in spectral EEG, water maze and passive avoidance studies, respectively, as we found earlier that scopolamine treatment at these doses induces spectral EEG abnormality and disrupts behavior in water maze and passive avoidance tasks (Riekkinen Jr. et al., 1990b, 1991b, 1993, 1996a; Björklund et al., 1996). In our previous study, metrifonate at 10 mg/kg improved water maze spatial navigation and at 30 mg/kg improved passive avoidance behavior of young scopolamine-pretreated and medial septal-lesioned rats, and at 10 and 30 mg/kg it improved passive avoidance but had no effect on water maze spatial navigation in 27-month-old rats (Riekkinen Jr. et al., 1996a). Therefore, the doses of 10 and 30 mg/kg were selected to study a possible synergistic effect between nimodipine and metrifonate in young scopolamine-pretreated or medial septal-lesioned and aged (26–28-month-old) rats, respectively, in these behavioral tests. Furthermore, we found previously that 10 mg/kg was a subthreshold dose of metrifonate for suppression of neocortical high-voltage spindles and reversal of scopolamine-induced spectral EEG abnormality (Björklund et al., 1996). This dose of metrifonate was, therefore, chosen to study the effects of combined nimodipine and metrifonate treatment in the modulation of neocortical EEG activity.

2.3. Surgery

Medial septal (A: 0.0 mm, M: 0.0 mm, D: −7.0 mm relative to the bregma) lesioning was made by passing an anodal DC current (2 mA, 5 s) via stainless steel electrodes. Controls were treated identically, but no current was applied. The rats were deeply anesthetized with Equithesin during the operations. All the rats that were used for EEG recordings were anesthetized with Equithesin and stainless-steel screw (0.5 mm diameter) electrodes were used as monopolar epidural recording electrodes (A: 2.0

Table 1
The effects of nimodipine and metrifonate on passive avoidance testing trial (72 h after training trial) latencies expressed in seconds

Drug:	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII	
Vehicle YC:	331 ± 20	345 ± 18	346 ± 23	349 ± 17	348 ± 20	343 ± 23	345 ± 18	(8)
Vehicle:		SCOP2	SCOP2	MS	MS	Aged	Aged	
		178 ± 78 [a]	161 ± 66 [a]	156 ± 44 [a]	197 ± 84 [a]	161 ± 102 [a]	142 ± 68 [a]	(9)
N3	324 ± 34	179 ± 42 [a]		154 ± 58 [a]		128 ± 76 [a]		
N10	315 ± 38	170 ± 49 [a]		151 ± 63 [a]		155 ± 45 [a]		
N30	322 ± 27	178 ± 46 [a]		152 ± 67 [a]		142 ± 56 [a]		
M10			243 ± 45 [ab]		258 ± 95 [a]			
M10N10			284 ± 66 [ab]		281 ± 69 [ac]			
M30							234 ± 68 [ad]	(10)
M30N3							240 ± 83 [ad]	(9)
M30N10							242 ± 69 [ad]	(10)
M30N30							198 ± 71 [a]	(8)

Group means ± are shown. Nimodipine and metrifonate (and respective vehicles) were given daily p.o. 30 min before testing and scopolamine was injected daily i.p. 35 min before testing. The drug doses are expressed as mg/kg. The number of rats was eight in each drug treatment group in groups I–VI. The number of rats in each drug treatment group in group VII is shown in parentheses. Abbreviations: M = metrifonate; MS = medial septal-lesioned; N = nimodipine; SCOP = scopolamine; YC = young vehicle-treated controls. [a] $P < 0.05$ vs. young vehicle-treated control rats; [b] $P < 0.05$ vs. scopolamine-treated rats; [c] $P < 0.05$ vs. medial septal-lesioned rats; [d] $P < 0.05$ vs. vehicle-treated aged rats.

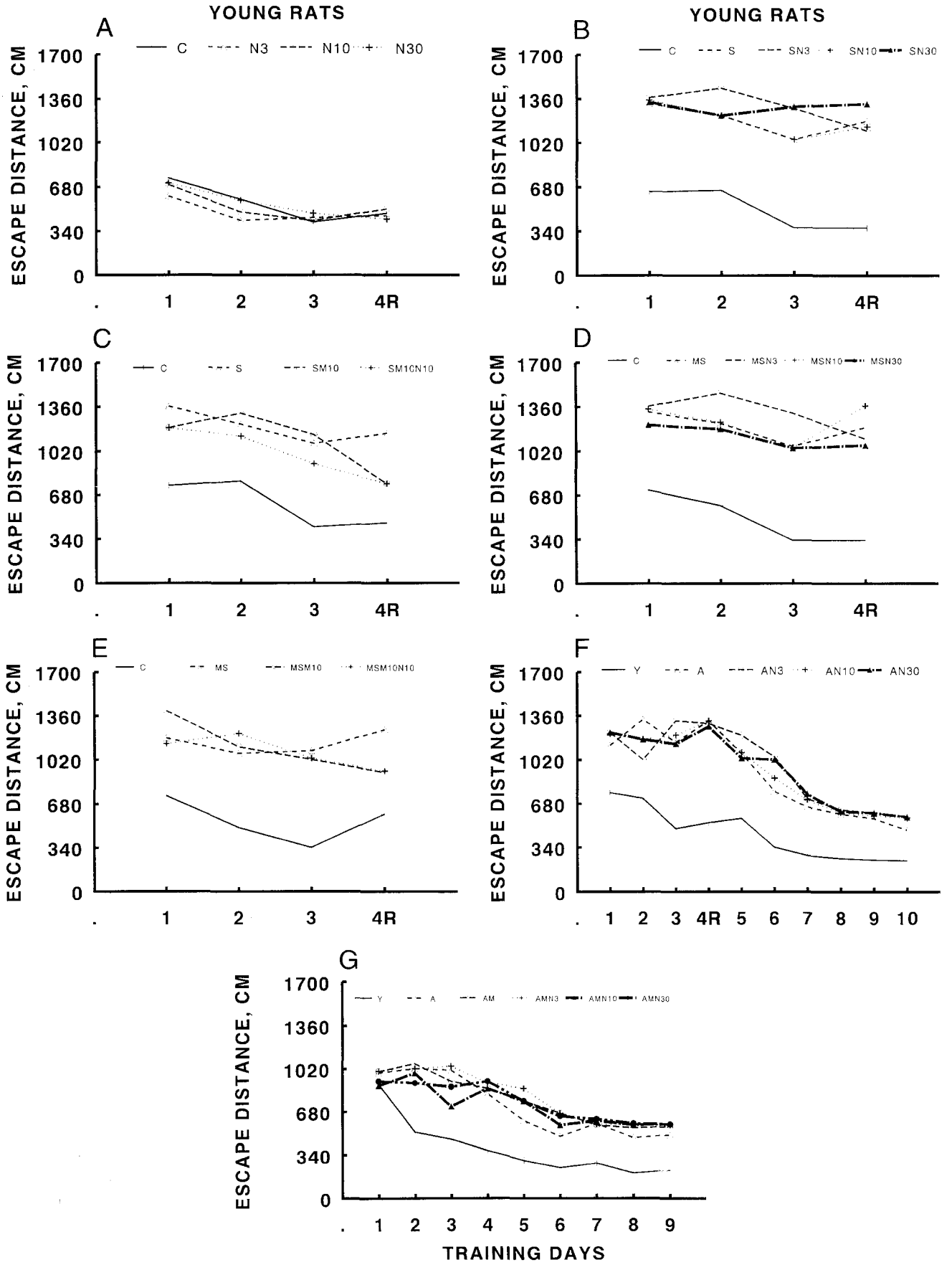
YOUNG RATS
A
C
N3
N10
N30
B
S
SN3
SN10
SN30
C
SM10
SM10N10
D
MS
MSN3
MSN10
MSN30
E
MSM10
MSM10N10
F
Y
A
AN3
AN10
AN30
G
AM
AMN3
AMN10
AMN30
ESCAPE DISTANCE, CM
1700
1360
1020
680
340
0
1
2
3
4R
5
6
7
8
9
10
TRAINING DAYS

Table 2
The effects of nimodipine alone and combined with a subthreshold dose of metrifonate on cortical high-voltage spindles and spectral power EEG in vehicle and scopolamine-treated rats

	Vehicle	N 3	N 10	N 30	M 10	N3 + M 10	N 10 + M10
HVS, s	100 ± 22	104 ± 37	96 ± 30	101 ± 35	105 ± 34	103 ± 34	107 ± 35
Vehicle	1170 ± 123	1140 ± 131	1199 ± 89	1167 ± 101	1198 ± 88	1180 ± 88	1201 ± 133
SCOP 0.2	1534 ± 121 [a]	1517 ± 103 [a]	1532 ± 120 [a]	1501 ± 142 [a]	1501 ± 122 [a]	1567 ± 145 [a]	1521 ± 133 [a]

The number of rats was eight. For high-voltage spindle recordings, a 3-day recovery period was allowed between the recordings, and for EEG spectrum recordings there were 4 day recovery periods between recordings. Group means ± S.D. of neocortical high-voltage spindles (HVS) in seconds during a cumulative 15 min period of waking immobility and 1–20 Hz sum amplitude values in μV are shown. Nimodipine and metrifonate were given p.o. 30 min before recording and scopolamine was injected i.p. 35 min before recording. Drug doses shown are mg/kg. Abbreviations: N = nimodipine; M = metrifonate; SCOP = scopolamine. Nimodipine alone or when combined with a subthreshold dose of metrifonate did not affect high-voltage spindles and could not alleviate the EEG abnormality (increase in 1–20 Hz spectral EEG values) caused by scopolamine treatment. [a] $P < 0.05$ vs. vehicle.

mm, L: ±2.5 mm relative to the bregma). Two additional screw electrodes were placed in the midline above the cerebellum and served as ground and indifferent electrodes. The screw electrodes and female pins were covered with dental acrylic cement.

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, 1996a). 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. Testing consisted of 4 consecutive days of testing. Five platform trials of 70 s were run per day during the first 3 training days. The platform location was kept constant (at the southwest quadrant) during this period of training. During the 4th day of training the location of the escape platform was reversed to the northeast quadrant and six trials of 50 s were run. On each trial (day 1–4), the rats were allowed to stay on the platform for 10 s. If the rats failed to find the platform during the maximum duration of the trial, the experimenter placed them on it for 10 s. A 30-s recovery period was allowed between training trials.

The water maze training schedule for the aged rats was different from that of the young rats as aged controls did not learn the task in 4 days. The design of the experiment investigating the effect of single daily drug treatment on spatial navigation was similar to that used to train young rats. However, after we had trained aged rats in water maze test for 4 days and measured passive avoidance

Fig. 1. The effects of nimodipine and metrifonate on water maze spatial navigation performance. The *x* axis shows the training days. The *y* axis shows the daily values (cm) for group mean escape distances. Nimodipine and metrifonate were given daily p.o. 30 min before testing and scopolamine was injected daily i.p. 35 min before testing. The doses are expressed as mg/kg. Abbreviations: C = controls (vehicle-treated); M = metrifonate; MS = medial septal-lesioned; N = nimodipine; S = scopolamine; A = aged rats; Y = young rats. A: the effects of nimodipine on water maze performance by young neurologically intact rats. Groups: controls, nimodipine 3, 10 and 30 mg/kg. The number of rats was eight in each group. Note the lack of effect of nimodipine on values for escape distances. B: the effects of nimodipine on water maze performance of scopolamine-treated rats. Groups: controls, scopolamine 0.4 mg/kg, scopolamine 0.4 mg/kg + nimodipine 3, 10 or 30 mg/kg. The number of rats was eight in each group. Nimodipine could not alleviate the water maze navigation defect caused by scopolamine treatment. C: the effects of combined nimodipine + threshold dose of metrifonate on water maze performance by scopolamine-treated rats. Groups: controls, scopolamine 0.4 mg/kg, scopolamine 0.4 mg/kg + metrifonate 10 mg/kg, scopolamine 0.4 mg/kg + metrifonate 10 mg/kg + nimodipine 10 mg/kg. The number of rats in each group was eight. Scopolamine impaired the water maze navigation, and nimodipine could not increase the beneficial effect of metrifonate. D: the effects of nimodipine on water maze performance of medial septal-lesioned rats. Groups: control-lesioned, medial septal-lesioned, medial septal-lesioned + nimodipine 3, 10 or 30 mg/kg. The number of rats in each group was eight. Nimodipine could not improve the performance failure induced by medial septal lesioning. E: the effects of combined nimodipine + threshold dose of metrifonate on water maze performance of medial septal-lesioned rats. Groups: control-lesioned, medial septal-lesioned, medial septal-lesioned + metrifonate 10 mg/kg, medial septal-lesioned + metrifonate 10 mg/kg + nimodipine 10 mg/kg. The number of rats in each group was eight. Medial septal lesioning-impaired water maze navigation; nimodipine could not increase the beneficial effect of metrifonate observed on the fourth day of testing. F: the effects of nimodipine on water maze performance of aged (26–28-month-old) rats. Groups: young control rats, aged control rats, aged rats + nimodipine 3, 10 or 30 mg/kg. The number of rats in each group was eight. The rats were at first trained for 4 days like the young animals (see Section 2). After a recovery period three additional training days were run (training day 5–7); the aged rats learned the test and did not improve markedly during the last three (training day 8–10) days. The age-related impairment of water maze navigation was not alleviated by short-term nimodipine treatment. G: the effects of combined nimodipine + subthreshold dose of metrifonate on water maze performance of aged rats. Groups: young control rats (eight), aged control rats (nine), aged rats + metrifonate 30 mg/kg (ten), aged rats + metrifonate 30 mg/kg + nimodipine 3 (nine), 10 (ten) or 30 (eight) mg/kg. The number in parentheses indicates the number of rats in each group. Note the lack of an effect of metrifonate alone and combined metrifonate and nimodipine treatment on water maze navigation by aged rats.

during the 5th and 6th day, a recovery period of 2 days was allowed. Following the recovery period 6 additional training days were run (8 × 50 s platform trials per day, 10 s on the platform, 50 s recovery period). The experiment on the effects of combined treatment with metrifonate + nimodipine on the age-induced water maze and passive avoidance defect had the following training schedule: during the first 9 days the platform location was kept constant and six 50 s trials (10 s on the platform, 50 s recovery period) were run per day. On the 10th and 11th day of behavioral testing, the passive avoidance study was done. For the description of treatment groups, see legend to Fig. 1.

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 s 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 test trial 72 h later the rat was again placed in the bright compartment, and the latency to enter the dark compartment was measured (360 s maximum latency). The study drugs were administered before the training trial as outlined in the legend to Table 1.

2.6. EEG recording system

We started the study recordings 7–10 days after surgery. For familiarisation with the recording environment, the rats were placed twice into this environment before the recordings were started. To ascertain that the high-voltage spindle levels would be constant, seven baseline sessions were run before the study recordings were started. No difference in high-voltage spindle activity was seen between the fifth, sixth and seventh baseline recording sessions (data not shown). To control for circadian variation in arousal level, recordings were made at the same time of the various recording days for individual animals. Recordings were made between 08:00–16:00. During recording the rats moved freely in their home cages and had a cable attached to their electrodes. The waking immobility (eyes open, head held up) (15 min cumulative period) -related high-voltage spindles (number and duration) were measured using a computerized EEG waveform analyzing system (Jäkälä et al., 1995). The computerized system was programmed to measure waking-immobility-related high-voltage spindle activity based on the amplitude (minimum twice as great as non-spindling activity), duration (minimum 1 s) and frequency (6–12 Hz) criteria described in detail earlier (Jäkälä et al., 1995). No movement of body parts or head (except mild tremor of vibrissae, head or jaw that often occur during high-voltage spindles) recorded by the magnetic coil binding of the EEG cable on the head of the rats was allowed 1 s before or during any of the high-voltage spindle epochs. In spectral EEG recordings, five 4 s artifact-free waking-immobility samples were selected by the person making the recording. The samples were digitized using a sampling rate of 250 Hz. Samples were measured simultaneously from both active recording sites. The spectral amplitude (μV) was analyzed from 1–4, 4–8, 8–12 and 12–20 Hz bands, and a sum amplitude of 1–20 Hz activity was also calculated, because we have shown earlier that scopolamine 0.2 mg/kg treatment increases both the amplitude values in all these different frequency bands and the 1–20 Hz sum amplitude values (Björklund et al., 1996). Following the baseline recordings the effects of nimodipine and metrifonate on high-voltage spindles and spectral EEG were tested as shown in Table 1.

2.7. Statistics

A one-way analysis of variance (one-way ANOVA) followed by Duncan's post-hoc multiple group comparison was used for the water maze and passive avoidance results. Wilcoxon and Mann-Whitney U tests were used for the EEG results.

3. Results

3.1. Intact young rats

3.1.1. Water maze and passive avoidance

Nimodipine 3, 10 and 30 mg/kg failed to facilitate water maze navigation, as escape distance values were not improved during the training period ($F(3,27) < 0.2$, $P > 0.05$ vs. controls, for all daily comparisons) (Fig. 1A). Furthermore, nimodipine, at any of the doses tested, did not affect passive avoidance training (data not shown) or testing trial performance ($F(3,27) < 0.1$, $P > 0.05$ vs. controls, for both comparisons) (Table 1).

3.1.2. Cortical EEG (Table 2)

Nimodipine 3, 10 or 30 mg/kg did not markedly affect high-voltage spindles or spectral EEG values (amplitudes) of any of the frequency bands (1–4, 4–8, 8–12, 12–20 Hz) or 1–20 Hz sum amplitude values ($P > 0.05$, for all comparisons). The combination of nimodipine 3 or 10 mg/kg and metrifonate 10 mg/kg did not significantly modulate high-voltage spindles or spectral EEG values of any of the above mentioned frequency bands or 1–20 Hz sum amplitude values ($P > 0.05$, for all comparisons).

3.2. Scopolamine-treated rats

3.2.1. Water maze

Scopolamine 0.4 mg/kg impaired water maze navigation during all the testing days as escape distance values

were increased ($F(4,44) > 7.0$, $P < 0.05$, for all daily comparisons) (Fig. 1B). Nimodipine 3, 10 or 30 mg/kg had no effect on the scopolamine treatment-induced increase in escape distance values during any of the test days ($P > 0.05$ vs. scopolamine group in all daily comparisons) (Fig. 1B). Single or combined treatment with metrifonate 10 mg/kg and metrifonate 10 mg/kg + nimodipine 10 mg/kg was ineffective to alleviate the scopolamine treatment-induced increase of escape distance values ($P > 0.05$ vs. scopolamine group) during the first 3 days of testing. However, on the 4th day of testing a significant alleviation of scopolamine treatment-induced water maze failure was observed with metrifonate 10 mg/kg and metrifonate 10 mg/kg + nimodipine 10 mg/kg ($P < 0.05$ vs. scopolamine group) (Fig. 1C).

3.2.2. Passive avoidance (Table 1)

Scopolamine 2 mg/kg had no effect on passive avoidance training trial behavior ($F(4,44) = 0.2$, $P > 0.05$) (data not shown). Testing trial performance, however, was impaired by scopolamine 2 mg/kg ($F(4,44) = 7.5$, $P < 0.05$). Nimodipine 3, 10 or 30 mg/kg had no effect on scopolamine treatment-induced passive avoidance failure ($P > 0.05$ vs. single scopolamine-treated rats, for all comparisons). Metrifonate 10 mg/kg and metrifonate 10 mg/kg + nimodipine 10 mg/kg-treated rats were impaired compared with the controls ($P < 0.05$ vs. controls), but these treatments also effectively alleviated the scopolamine treatment-induced passive avoidance failure ($P < 0.05$ vs. scopolamine group).

3.2.3. Cortical EEG (Table 2)

Scopolamine 0.2 mg/kg increased the amplitudes of all individual frequency bands (1–4, 4–8, 8–12, 12–20 Hz) and sum amplitude of 1–20 Hz spectral EEG values ($P < 0.05$). Nimodipine 3, 10 and 30 mg/kg did not alleviate the EEG abnormality caused by scopolamine treatment ($P > 0.05$). Combination of a subthreshold dose of metrifonate (10 mg/kg) with nimodipine 3 or 10 mg/kg did not significantly alleviate the EEG abnormality following scopolamine administration ($P > 0.05$).

3.3. Medial septal-lesioned rats

3.3.1. Water maze

Medial septal lesioning impaired water maze navigation during all test days, as the escape distance values were increased (nimodipine study: $F(4,34) > 5.9$; nimodipine + metrifonate study: $F(3,27) > 6$, $P < 0.05$ vs. sham-lesioned rats, for all daily comparisons) (Fig. 1, parts D and E). Nimodipine 3, 10 or 30 mg/kg had no effect on escape distance values of lesioned rats as measured during the 4 day test period ($P > 0.05$) (Fig. 1D). Single metrifonate 10 mg/kg or a combination of metrifonate 10 mg/kg + nimodipine 10 mg/kg had no marked effect on the lesioning-induced increase in escape distance values measured during the first 3 training days ($P > 0.05$) (Fig. 1E). However, on the 4th day of testing, metrifonate 10 mg/kg alone or combined metrifonate 10 mg/kg + nimodipine 10 mg/kg as effectively improved water maze navigation of lesioned rats ($P < 0.05$) (Fig. 1E).

3.3.2. Passive avoidance (Table 1)

Lesioning had no effect on passive avoidance training trial values ($F(4,44) = 0.7$, $P < 0.05$) (data not shown), but impaired the passive avoidance testing trial performance, as the re-entry latencies were shorter than the re-entry values of sham-operated rats ($F(4,34) = 12.1$, $P < 0.05$). Nimodipine 3, 10 or 30 mg/kg failed to alleviate the passive avoidance failure of lesioned rats ($P > 0.05$). The lesioned rats treated with metrifonate 10 mg/kg alone or the combination of metrifonate 10 mg/kg + nimodipine 10 mg/kg tended to perform better than the vehicle-treated lesioned rats. This difference was significant only for the combination treatment ($P < 0.05$ vs. vehicle-treated lesioned rats), but no difference was found between the groups treated with metrifonate, 10 mg/kg alone, or in combination with nimodipine 10 mg/kg ($P > 0.05$).

3.4. Aged rats

3.4.1. Nimodipine alone

3.4.1.1. Water maze. Aged rats were impaired in the water maze navigation during the 1st, 2nd, 3rd and 4th days of training ($F(4,34) > 9$, $P < 0.05$, for all daily comparisons) (Fig. 1F). Nimodipine 3, 10 and 30 mg/kg had no effect on the escape distance values of aged rats ($P > 0.05$) (Fig. 1F). As the aged rats were still markedly impaired during the 4th test day, additional training was started and was continued for 6 days (see Section 2). The aged rats were still impaired during the next 6 training days ($F(4,34) > 8$, $P < 0.05$; for all daily comparisons), and none of the nimodipine doses examined had any improving effect on water maze performance ($P > 0.05$) (Fig. 1F).

3.4.1.2. Passive avoidance (Table 1). All the groups of aged rats (saline, nimodipine 3, 10 and 30 mg/kg) performed well during the training trial ($F(4,34) = 0.2$, $P > 0.05$, data not shown), but their performance was impaired compared with that of the young rats during the test trial ($F(4,34) = 12.1$, $P < 0.05$).

3.4.2. Nimodipine + metrifonate

3.4.2.1. Water maze. The performance of all groups of aged rats (saline, metrifonate 30 mg/kg, metrifonate 30 mg/kg + nimodipine 3, 10 or 30 mg/kg) was impaired compared with that of young rats during the 9 day test period ($F(5,47) > 10$, $P < 0.05$, for all daily comparisons) (Fig. 1G). A comparison of escape distance values of different groups of aged rats showed no overall group effect on any of the test days ($P > 0.05$) (Fig. 1G).

3.4.2.2. Passive avoidance (Table 1). All the groups of aged rats (saline, metrifonate 30 mg/kg, metrifonate 30 mg/kg + nimodipine 3, 10 or 30 mg/kg) performed well during the training trial ($F(5,47) = 0.1$, $P > 0.05$; data not shown), but had an impaired performance in the passive avoidance testing trial ($F(5,47) = 13.1$, $P < 0.05$). Metrifonate treatment at a dose of 30 mg/kg facilitated passive avoidance responding of aged rats ($P < 0.05$). Nimodipine at doses of 3 or 10 mg/kg did not affect the improving effect of metrifonate treatment on passive avoidance behavior ($P > 0.05$). On the contrary, the highest dose of nimodipine reduced to some extent the effect of metrifonate on the age-related impairment of passive avoidance behavior, as the group treated with metrifonate 30 mg/kg + nimodipine 30 mg/kg did not do significantly better than the control aged rats ($P > 0.05$ vs. aged saline-treated rats).

4. Discussion

We now found that short-term nimodipine treatment had no effect on water maze and passive avoidance behavior of young neurologically intact, scopolamine-pretreated and medial septal-lesioned rats, or aged rats. Furthermore, in none of our models used in the present study did nimodipine enhance or diminish the slight beneficial effect of a low dose of metrifonate. Finally, nimodipine did not markedly affect neocortical high-voltage spindles or cortical EEG slowing following scopolamine, and could not make active a subthreshold dose of metrifonate.

Different theories could explain the lack of effect of nimodipine on passive avoidance and water maze behavior. Firstly, the near-perfect performance of young adult control rats in our passive avoidance study may have created a ceiling effect upon which no drug treatment-induced improvement could be detected. A second possibility is that we did not use an optimal dose of nimodipine in our studies. Quartermain et al. (1993) found that treatment with nimodipine at a number of different doses (0.5, 1, 2.5, 5 and 10 mg/kg) only non-significantly improved passive avoidance behavior in mice and the best dose of nimodipine was 0.5 mg/kg. Deyo (1990) reported that nimodipine at 1 mg/kg facilitated and at 5 mg/kg impaired visual discrimination behavior in chicks. Therefore, the possible improving effects of short-term nimodipine treatment on memory functioning may occur only over a narrow dose range. Yet, another possibility is that nimodipine has no marked effect on spatial and avoidance behaviors in young neurologically normal rats.

Indeed, the failure of nimodipine to alleviate the water maze and passive avoidance performance deficit caused by scopolamine treatment and medial septal lesioning also supports the hypothesis that short-term treatment with a dihydropyridine Ca^{2+} channel blocker does not markedly facilitate spatial and avoidance behavior. These findings also suggest that functional defects caused by blockade of muscarinic acetylcholine receptors or damage to the septohippocampal system cannot be alleviated by short-term nimodipine treatment. The water maze acquisition failure induced by scopolamine 0.4 mg/kg was reported to be reversed by another non-cholinergic therapy, i.e., d-cycloserine (1.0 mg/kg), a partial agonist at the glycine-B binding site on *N*-methyl-D-aspartate receptor complex (Pitkänen et al., 1995), suggesting that the doses of scopolamine used in the present study were not too high. The lack of an effect of nimodipine treatment on water maze and passive avoidance failure caused by medial septal lesioning is difficult to interpret simply as failure to activate the functioning of cholinergic cells of the septohippocampal system. Indeed, many non-cholinergic systems are non-specifically destroyed by electrolytic lesioning. For example, the γ-aminobutyric acid (GABA)-ergic cells of the septum that innervate and regulate the function of the hippocampus, and a number of fiber systems penetrating the lesioned septal area are destroyed (Dunnett et al., 1991; Dunnett and Fibiger, 1993).

The present study could also show no effect of short-term nimodipine treatment on age-related water maze and passive avoidance performance failure, further suggesting that nimodipine at the doses used has no acute positive therapeutic effect on water maze and passive avoidance memory functioning in aged rats either. However, there are previous reports that short-term treatment with nimodipine over a narrow dose range significantly improved acquisition and memory retrieval of aged primates and rabbits (Deyo et al., 1989; Sandin et al., 1990). Furthermore, food-motivated spatial working memory in the radial-arm maze was facilitated by short-term nimodipine treatment in aged Long-Evans Hooded rats (LeVere and Walker, 1991). Thus, since we tested Wistar rats in aversively motivated reference memory tests, it is possible that the effect of nimodipine may depend on the mnemonic demands of the test, the rat strain used and/or differing pharmacokinetics (blood and/or brain levels) of nimodipine between different animal species and/or rat strains.

The cholinesterase inhibitor, metrifonate, at 10 mg/kg partially alleviated the scopolamine- or medial septal lesioning-induced water maze and passive avoidance deficits in young rats, and at 30 mg/kg partially alleviated the passive avoidance failure but had no effect on the water maze spatial navigation deficit exhibited by aged (26–28-month-old) rats. These present results are in good agreement with the results of our previous study (Riekkinen Jr. et al., 1996a). In that study, metrifonate at 10–100 mg/kg improved water maze spatial navigation and at 10 mg/kg non-significantly and at 30–100 mg/kg significantly improved passive avoidance behavior of young scopolamine-pretreated and medial septal-lesioned rats (Riekkinen Jr. et al., 1996a). It is relevant that, both in the present and in our previous study, the improvement in water maze performance of young scopolamine-pretreated and medial septal-lesioned rats by metrifonate treatment was more

pronounced during the platform reversal stage than during the reference (fixed location of the hidden escape platform) memory stage. However, as the metrifonate treatment was administered during all the training days it is difficult to interpret the improved performance during the platform reversal stage in terms of improved working memory function. Indeed, metrifonate-treated rats may have adopted a different navigation strategy during the fixed platform stage (the first 3 training days) that they could more flexibly use as an alternative during the platform reversal stage (the 4th training day) than scopolamine-pretreated or lesioned rats receiving vehicle treatment. Furthermore, metrifonate may also have simply increased the speed of extinction of previously learned spatial navigation strategy, leading to a better escape performance during the platform reversal stage.

Previously, metrifonate at 10 and 30 mg/kg partially alleviated the passive avoidance failure but had no effect on water maze spatial navigation in 27-month-old rats, and in slightly younger (23-month-old) rats it at 10 and 30 mg/kg partially alleviated the water maze spatial navigation deficit and at 30 mg/kg partially alleviated the passive avoidance deficit exhibited by these rats (Riekkinen Jr. et al., 1996a). In line with these results, metrifonate 30 mg/kg could not alleviate the water maze spatial navigation reference or working memory deficits exhibited by 26–28-month-old rats in the present study. Thus, it is possible that neuropathological deficits develop during advanced aging (Decker and McGaugh, 1991; Miettinen et al., 1993) and aggravate the spatial navigation failure and thereby mask the beneficial effect of metrifonate observed in younger, 23-month-old, rats. However, 26–28-month-old rats were chosen for the present study because, based on the results of our previous study (Riekkinen Jr. et al., 1996a), we expected them to have become more sensitive to a cholinergic treatment if another age-related pathology, i.e., the Ca^{2+} overload, were treated concomitantly.

Brain cholinesterase (ChE) may not be markedly inhibited at the behaviorally effective doses of metrifonate (10 and 30 mg/kg). Based on ex vivo experiments, weak ChE inhibition could occur at best at 10 and 30 mg/kg. Indeed, ChE inhibition may be induced at higher doses. Metrifonate has an ED_{50} of about 90 mg/kg for ChE inhibition (Hinz et al., 1995; Van der Staay et al., 1996b). Doses up to 25 mg/kg did not induce significant (> 20%) ChE inhibition. Different explanations, which have been discussed in detail previously (Van der Staay et al., 1996a,b), have been suggested to explain this discrepancy between the ex vivo and the behavioral data. Briefly, upon repeated administration of metrifonate, the degree of ChE inhibition could significantly accumulate (Dubois and Cotter, 1995; Chan and Peters, 1989) due to metrifonates characteristics as a prodrug for the irreversible ChE inhibitor, dichlorvos (Hinz et al., 1996a). Under physiological conditions this is a slow process, so that the resulting ChE inhibition is kinetically protracted as opposed to the situation observed with conventional ChE inhibitors, including, e.g., tetrahydroaminoacridine (Hinz et al., 1996a). Alternatively, metrifonate might act via a mechanism other than ChE inhibition, although there is no direct evidence for this explanation. Indeed, the side-effect profile of metrifonate is exclusively cholinergic (Blokland et al., 1995), and the receptor binding profile of metrifonate (assessment of more than 40 biological targets) does not suggest any additional drug effect (Hinz et al., 1996b). Furthermore, in a microdialysis study only weak increases in brain monoamine levels when compared with strong dose-dependent increases in acetylcholine levels were seen after systemic metrifonate treatment (Mori et al., 1995). Metrifonate at 20, 40 and 80 mg/kg (s.c.) in a dose-dependent manner significantly increased cortical acetylcholine levels, whereas only 80 mg/kg of systemic metrifonate could increase the levels of noradrenaline. However, the dopamine levels were significantly increased by lower doses of systemic metrifonate (20 mg/kg). Finally, it could also be hypothesized that even minor increases in brain ChE inhibition could be sufficient to improve cognitive performance as suggested, e.g., by Nordgren et al. (1992). Altogether, based on the data available, it is not possible to choose between these alternative explanations. It is, however, interesting that in a previous study metrifonate, and less clearly dichlorvos, facilitated the acquisition of a standard version of the Morris water maze task at 10 and 30 mg/kg, which may induce only modest inhibition of ChE, whereas other structurally unrelated ChE inhibitors E2020, physostigmine and tetrahydroaminoacridine, although significantly inhibiting brain ChE could not improve acquisition of the water maze task (Van der Staay et al., 1996b). This suggested that ChE inhibition might not be the only mechanism involved in the cognition-enhancing properties of metrifonate and related drugs, at least in young intact rats.

Because the doses of 10 and 30 mg/kg were at the lower end of the dose range of metrifonate, which in our previous study had beneficial effects in these behavioral models, we selected these doses for the present study to allow detection of possible negative or positive modulatory effects by nimodipine on the beneficial response to acute metrifonate treatment. However, the performance-improving effects of metrifonate on water maze and passive avoidance behaviors at these selected low threshold doses were not significantly modulated by short-term nimodipine treatment. Firstly, the combination of a threshold dose of metrifonate 10 mg/kg and nimodipine 10 mg/kg produced an effect similar to that of metrifonate 10 mg/kg alone in scopolamine-pretreated and medial septal-lesioned rats in the water maze and passive avoidance tests in young rats. Secondly, the effects of combined treatment with metrifonate 30 mg/kg and nimodipine 3 or 10 mg/kg failed to show any synergistic effect on water maze and passive avoidance behavior of aged rats, whereas nimodipine at one high dose (30 mg/kg) partially reduced the beneficial effect of metrifonate on age-related failure of

passive avoidance behavior. However, the lack of significant modulatory effects of nimodipine on the beneficial response to metrifonate treatment suggests that short-term treatment with drugs blocking L-type Ca^{2+} channels and cholinesterase inhibitors does not synergistically stimulate cognitive functions.

There are no data available regarding pharmacokinetic interactions between short-term nimodipine and metrifonate treatments. However, it is unlikely that nimodipine would affect the bioavailability of metrifonate as the behavioral and neurophysiological effects of metrifonate were not altered by short-term nimodipine treatment in the present study.

Nimodipine also had no effect on cortical EEG arousal since we found no effect on spontaneously occurring thalamocortically generated neocortical high-voltage spindles or spectral EEG in young neurologically normal rats, nor did nimodipine alleviate the EEG spectrum slowing induced by scopolamine (0.2 mg/kg) treatment. Neocortical high-voltage spindles reflect oscillatory activity in thalamocortical networks and occur typically only during low arousal and low vigilance states. During neocortical high-voltage spindles, rhythmically active γ-aminobutyric acid-containing nucleus reticularis thalamus neurons phasically hyperpolarize their thalamocortical target neurons, and in the absence of other depolarizing inputs, voltage- and time-dependent rebound Ca^{2+} spikes occur in thalamocortical relay neurons in a phase-locked manner (McCormick, 1990, 1992; Steriade et al., 1993). In the present study, acute nimodipine administration could not decrease high-voltage spindles in young rats, suggesting that blockade of voltage-dependent L-type Ca^{2+} channels by systemic, acute nimodipine treatment cannot effectively modulate information processing in thalamocortical networks.

Scopolamine 0.2 mg/kg, as found previously (Riekkinen Jr. et al., 1991b, 1993; Björklund et al., 1996), induced slow wave activity, i.e., increased the sum amplitude values of 1–20 Hz band in spectral EEG activity. This probably reflects a firing rate of cortical pyramidal cells decreased by scopolamine treatment (McCormick, 1990, 1992; Steriade et al., 1993). Nimodipine did not modulate spectral EEG activity of young intact rats and could not counterbalance the effect of scopolamine on spectral EEG activity, suggesting that acute nimodipine treatment cannot effectively modulate cortical EEG arousal. Finally, the combination of nimodipine 3 or 10 mg/kg with a subthreshold dose of metrifonate 10 mg/kg, could not suppress neocortical high-voltage spindles or scopolamine-induced spectral EEG abnormality. Previously, metrifonate at higher doses (30–60 mg/kg) suppressed spontaneously occurring high-voltage spindles in young and aged rats, and at 30–100 mg/kg it fully restored normal spectral EEG in scopolamine-treated rats (Björklund et al., 1996). Therefore, if nimodipine and metrifonate had synergistic effects this should have become evident in the present study.

The present results are important as they indicate that blockade of L-type Ca^{2+} channels does not mask the memory-improving effect of treatment with a cholinesterase inhibitor, metrifonate. According to the Ca^{2+} overload hypothesis (Khachaturian, 1984), the degeneration of neurons is mediated by critical factors such as time and Ca^{2+} levels. Therefore, the more relevant therapeutic effect of Ca^{2+} antagonists such as nimodipine may be to prevent the Ca^{2+} overload in neurons over long periods of time and thereby protect neurons against insults such as those from excitotoxins, stroke, ischemia or trauma (Levy et al., 1992). Indeed, a recent study found that chronic nimodipine treatment prevented dendritic atrophy in aged rabbits (Mervis, 1993) and stimulated memory in aged rats. Furthermore, long-term nimodipine treatment resulted in improvement of sensorimotor behavioral functions in aged rats (Schuurman et al., 1987), and reduced the age-dependent loss of synapses of granular cells in the dentate gyrus of the hippocampus (De Jong et al., 1992). The present findings showing that short-term nimodipine treatment does not block the propensity of metrifonate to improve cognitive performance in rats leave still open the possibility that chronic nimodipine treatment via its proposed neuroprotective actions and stabilization of neuronal Ca^{2+} homeostasis might slow down the age-related decline of cognitive functions and cortical EEG arousal, and add to the therapeutic effects of metrifonate. Indeed, 4 month nimodipine treatment decreased cortical high-voltage spindles in aged rats, and the threshold dose of metrifonate for suppression of high-voltage spindles was significantly lower in nimodipine-treated than in placebo-treated aged rats (30 mg/kg vs. 60 mg/kg p.o.), suggesting that chronic nimodipine treatment adds to the therapeutic effects of metrifonate to restore normal cortical electrical arousal in aged rats (Riekkinen Jr. et al., 1996b). We are continuing the investigation of the effects of combined chronic nimodipine and metrifonate treatment on performance in animal cognition models.

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