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Research report

Effect of chronic nimodipine on spatial learning and on long-term potentiation

Kimberley A. Kane *, Gilbert B. Robinson

Psychology Department, University of New Brunswick, Fredericton, NB, E3B 6E4, Canada

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Abstract

The present study examined the effect of nimodipine on a reference memory task and on the induction and maintenance of long-term potentiation (LTP) in the hippocampal dentate gyrus. Young rats, subcutaneously implanted with either a 30 mg nimodipine or placebo pellet, were trained on the Barnes circular platform task. Retention was tested 15 days following acquisition. Following behavioural testing, recording and stimulating electrodes were implanted in the granule cell layer of the dentate gyrus and the perforant path, respectively. Pre-pellet baseline evoked potentials were collected. Nimodipine or placebo pellets were again subcutaneously implanted, according to the original groupings, and post-pellet baseline evoked potentials were obtained. LTP was then induced in the granule cell population by perforant path tetanization and the decay of LTP was followed for 15 days. Nimodipine significantly decreased the number of trials to reach both the acquisition and the retention criterion on the circular platform task, but did not alter granule cell excitability, LTP threshold, or the magnitude of LTP. Sustained nimodipine administration, however, increased the decay rate of LTP of the population spike, but did not affect the decay rate for LTP of the EPSP. No significant correlations were obtained between behavioural and electrophysiological measures. These results provide further evidence against a simple direct relationship between LTP and spatial learning. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Nimodipine; Calcium; LTP; Spatial memory; Hippocampus

1. Introduction

Nimodipine is one of a number of dihydropyridine, L-type calcium channel antagonists [16]. Unlike other dihydropyridines, nimodipine is effective on the central nervous system (CNS) at dosages which are ineffective on peripheral circulation [17]. As a result, there has been a great deal of interest in potential beneficial effects of nimodipine on CNS function. Several lines of investigation, for example, have shown that nimodipine administration is beneficial in brain dysfunction associated with trauma (e.g. stroke) or aging (e.g. cognition) [1,13,18,27].

The beneficial effects of nimodipine are not, however, limited to aged or neurologically impaired animals. A number of studies have demonstrated improved hippocampal-dependent associative learning in young, neurologically intact animals administered nimodipine [10,19,21]. Nimodipine administration, however, appears to have differential effectiveness on two spatial memory tasks. Sustained nimodipine administration enhanced acquisition of an 8-arm working memory task [19], but either impaired performance [20] or had no effect on initial acquisition or reversal learning of a water maze task [21]. Nevertheless, in the latter study,

* Corresponding author. Present address: Rotman Research Institute of Geriatric Care, Baycrest, Centre, University of Toronto, North York, Ontario, Canada, M6A 2E1. Fax: +1 416 7852862; e-mail:kimber@psych.utoronto.ca

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during reversal learning probe trials nimodipine treated rats did spend significantly more time than control animals in the quadrant that previously contained the goal platform. It is possible that the differential effectiveness of nimodipine administration on acquisition of the two spatial memory tasks reflects, in part, the different memory requirements of the two tasks.

To further investigate the effects of nimodipine on spatial learning, the present study examined the effects of chronic nimodipine administration in young rats on both the acquisition and retention of the Barnes circular platform reference memory task [2]. In addition, the effect of chronic nimodipine administration on long-term potentiation (LTP) in the hippocampal dentate gyrus was examined. Previous results demonstrated that nimodipine administration facilitates LTP induction in the hippocampal dentate gyrus [7]. Furthermore, there appears to be a positive correlation between LTP, a putative neural learning/memory mechanism, and spatial learning on the circular platform task [2,4] that may not exist for other spatial learning tasks [3]. Thus, the present study is a within animal comparison of the effects of chronic nimodipine administration on spatial learning and on LTP. Preliminary results have been presented elsewhere [15].

2. Materials and methods

2.1. Subjects

Twenty-eight male Long-Evans hooded rats, 3–4 months old, were housed individually and maintained on a 12-h light, 12-h dark cycle. Rats were handled daily and provided with free access to food and water. All testing was conducted in the first half of the light cycle.

2.2. Experimental protocol

2.2.1. Behavioural assessment

Rats were tested on the Barnes circular platform reference memory task [2]. The circular platform (1.2 m in diameter) had 18 holes, 9 cm in diameter, equally spaced around the periphery. A $41.8 \times 9.5 \times 10.1$ cm escape tunnel was located under one of the platform holes. The position of the tunnel was randomly determined for each animal and remained in a constant position, relative to distal cues (e.g. posters, chairs, mobiles, etc.), throughout testing. A 500 W halogen light was mounted 1.04 m above the platform. To eliminate light/shadow cues, a black curtain was hung from the edge of the platform to the floor. The surface of the platform was thoroughly washed after each trial to eliminate possible olfactory cues.

Prior to behavioural testing, rats were deeply anesthetized (65 mg/kg sodium pentobarbitol, i.p.) and a 30-day slow-release pellet containing either 30 mg nimodipine (manufactured from powdered nimodipine by Innovative Research, FL; $n = 14$) or placebo ($n = 14$), was subcutaneously implanted in the nape of the neck. This dose has effects on both behavioural [19] and electrophysiological [29] responses, without altering peripheral circulation [17].

Three days following pellet implantation, animals were given an adaptation trial (i.e. placed in their respective goal tunnel for 3 min). On subsequent days, animals received two trials per day with an inter-trial interval of 1 min. On each trial, animals were placed in a centrally located start box for 30 s. A pulley system then lifted the box from the platform. Rats were allowed a maximum of 3 min to locate the goal tunnel. If the tunnel was not found, the animal was placed beside the hole leading to the tunnel, allowed to descend, then to remain in the goal tunnel for 1 min. Training continued to an acquisition criterion of five consecutive trials with no more than three errors per trial. An error was scored if a rat's nose dropped below the surface of the platform into a hole that did not contain the goal tunnel. The number of errors and the time taken to find the escape tunnel were recorded for each trial.

Memory for the tunnel location was evaluated 15 days after the acquisition criterion was met. When retention performance was based on a single trial there was little variability between the two groups. Therefore, in subsequent experiments, nimodipine ($n = 6$) and placebo ($n = 7$) animals were given two trials/day until a retention criterion of three consecutive trials with no more than three errors per trial was met. The retention criterion was less stringent than the acquisition criterion to ensure that testing was completed within 30 days of pellet implantation.

2.2.2. Electrophysiological assessment

Two to three weeks after behavioural testing, animals were anaesthetized with 65 mg/kg sodium pentobarbitol (i.p.) and placed on a Kopf stereotaxic apparatus. The initial pellets were removed. A recording electrode (a single Teflon-coated stainless steel wire, 0.28 mm diameter) was lowered into the granule cell layer of the hippocampal dentate gyrus, 3.8 mm posterior and 2.3 mm lateral to bregma. A stimulating electrode (two Teflon-coated stainless steel wires twisted together and separated horizontally at the tips) was lowered into the perforant path at 7.8 mm posterior and 4.4 mm lateral to bregma. Final electrode sites were optimized using standard electrophysiological techniques. Electrodes were cemented in place and attached to a standard 9-pin connector. Electrophysiological testing began 1 week later.

First, pre-drug input/output (I/O) responses were determined. For each I/O, 30 electrical pulses (Grass S48 stimulator coupled to a Grass SIU5 stimulus isolation unit) were applied to the perforant path at 0.1 Hz. The granule cell response was amplified (Grass P15 preamplifier; bandpass 1 Hz–3 kHz), sampled at 10 kHz, and saved to disk. The stimulation intensity was increased after every tenth pulse. The first of the three intensities, which were chosen individually for each rat, was set at population spike threshold (range 2.5–10.0 mV). The second intensity was set at the midpoint between the first and third intensities. The third intensity was set to evoke the maximum amplitude population spike (range 9.0–20.0 mV). The pre-drug I/O response was determined once per day for three consecutive days.

Animals then were reimplanted with either nimodipine or placebo pellets, according to their behavioural group assignments. The pellets were reimplanted on the side of the neck opposite to the first pellet site. Following three days of recovery, post-drug I/O responses were obtained once per day for 3 days.

On the day following the third post-drug I/O, the threshold intensity for LTP induction was determined. Every 22 min, a set of 10 high-frequency trains (400 Hz, 50 ms) was applied to the perforant path. The intertrain interval was 5 s. Each train set was applied at one of five different train intensities, in an ascending series, from subthreshold for spike discharge to the intensity that evoked the maximum amplitude spike. An I/O was obtained 15–20 min after each train set. On each of the following 4 days, 10 trains (400 Hz, 50 ms, intertrain interval of 5 s) were applied at that intensity which evoked the maximum amplitude population spike to a single pulse. I/O responses were obtained at 15 min and 1, 2, 5, 8 and 15 days following the last train set. Decay constants were calculated for each animal at each of the three I/O intensities.

2.3. Statistical analyses

Behavioural data were analyzed using a 2-factor between group ANOVA.

The slope during the initial ms of positivity was used to monitor changes in the EPSP. The population spike amplitude was measured from the peak of the negativity to a tangent connecting spike onset and offset. LTP threshold was taken to be that train intensity which resulted in a population spike and EPSP amplitude, at each of the three intensities, that was 2 SEM larger than the average post-pellet/pre-tetanus baseline amplitudes.

Electrophysiological measures were evaluated by a 2×5 mixed-design analysis of variance (ANOVA). Correlations between electrophysiological measures and behavioural data were determined using Pearson product moment correlation coefficients. Statistical significance was set at $P < 0.05$.

3. Results

3.1. Behavioural results

On the first acquisition trial, the nimodipine group made an average of 9.9 ± 1.6 errors and took 128.6 ± 14.7 s to locate the goal tunnel. The control group made an average of 8.8 ± 1.6 errors and took 131.4 ± 17.6 s to find the goal tunnel on the first trial. Neither the number of errors nor the time taken to locate the goal tunnel differed significantly between groups. It therefore appears that nimodipine administration did not alter either motivational levels or motor ability.

Rats administered nimodipine required an average of 9.5 ± 0.6 trials to reach acquisition criterion compared to 13.5 ± 1.2 trials for rats administered placebo (Fig. 1). This difference was significant ($F(1,26) = 8.90$; $P < 0.01$).

The number of errors on the last five trials were examined to ensure that both groups had learned the task to an equal extent. The nimodipine group averaged 1.1 ± 0.6 errors per trial compared to 1.4 ± 0.8 errors for the placebo group. The difference was not significant.

As noted earlier, retention was initially tested with a single trial. However, the number of errors made by the two groups on the single retention trial was not significantly different. Thus, subsequent animals received more extensive retention training. These latter rats were

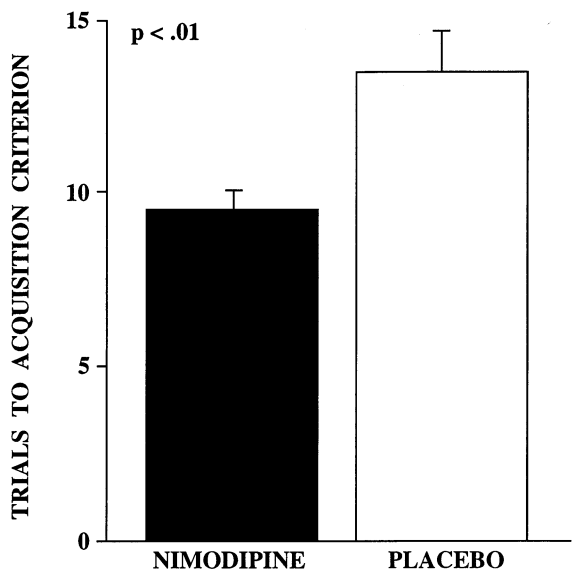

Fig. 1. Mean number of trials for acquisition of the Barnes circular platform task. Rats administered nimodipine required significantly ($P < 0.01$) fewer trials to acquire the task than did rats administered placebo.

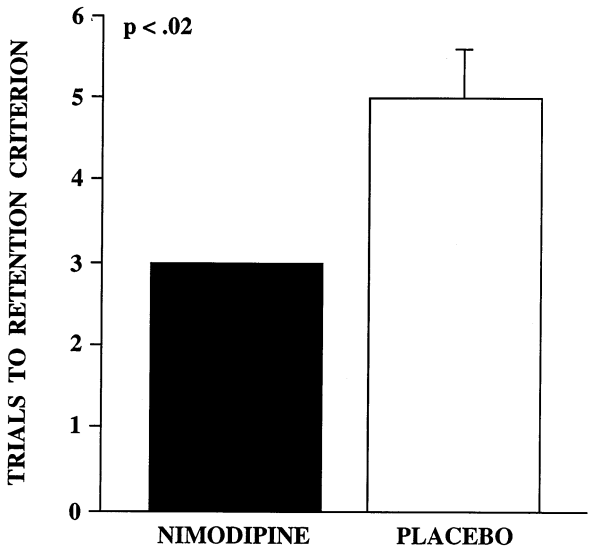

Fig. 2. Mean number of trials for rats to attain the retention criterion. Retention training began 15 days following acquisition. Rats administered nimodipine required significantly ($P < 0.02$) fewer trials to attain the retention criterion than did rats administered placebo.

not significantly different from the earlier rats with regards to trials to acquisition criterion or errors made on the first retention trial. Fig. 2 illustrates that sustained nimodipine administration significantly ($F(1,11) = 7.90$; $P < 0.02$) decreased the number of trials required to meet the retention criterion from 5.0 ± 0.6 for the placebo group to 3.0 ± 0.0 for the nimodipine group.

3.2. Electrophysiological results

Ten animals from the nimodipine group and nine animals from the control group were included in the electrophysiological studies. The remaining animals were excluded due either to computer failure (and subsequent loss of data) or to loss of acceptable electrophysiological responses following surgical recovery. The subset of animals included in the electrophysiological testing were not significantly different from their respective groups on either the acquisition or retention measures of learning.

Nimodipine administration had no significant effect on the baseline population spike or EPSP amplitude (Fig. 3) or the threshold intensity required to induce LTP of either the population spike or the EPSP. Furthermore, as shown in Fig. 3, the magnitude of LTP of both the population spike and the EPSP was not significantly altered by nimodipine administration.

As Fig. 4a demonstrates, LTP of the population spike decayed significantly faster in animals adminis-

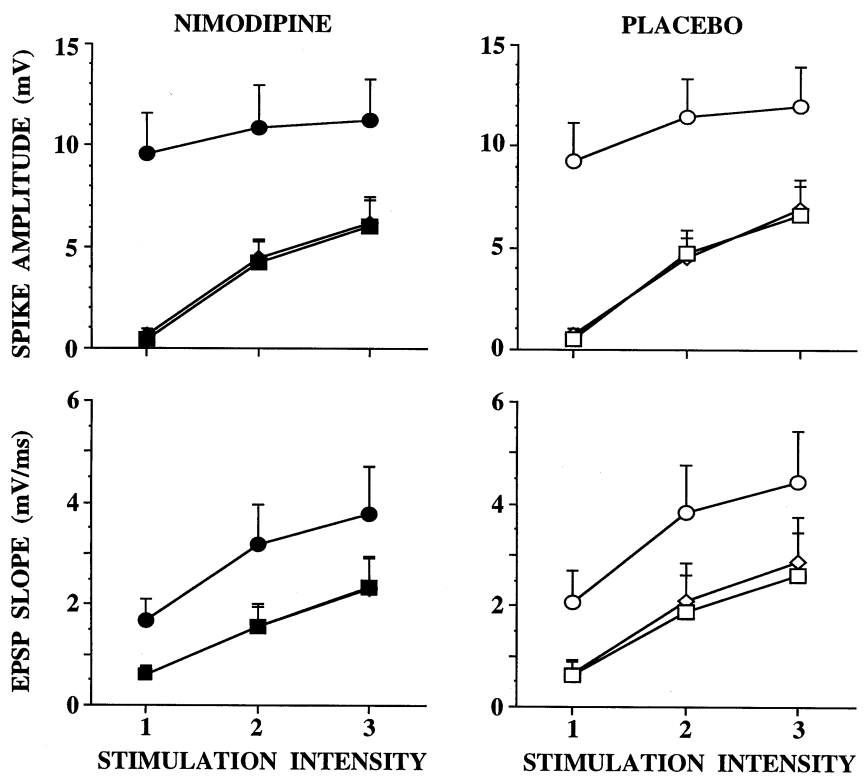

Fig. 3. Perforant path-granule cell input/output (I/O) responses obtained immediately prior to implantation of the nimodipine pellet (squares), 3 days following implantation of the nimodipine/placebo pellet (diamonds), and 15 min following perforant path tetanization (circles). Neither nimodipine nor placebo administration resulted in a significant change in the I/O response. Following perforant path tetanization, both groups exhibited significant LTP of both the population spike and EPSP. Nimodipine did not have a significant effect on the magnitude of LTP.

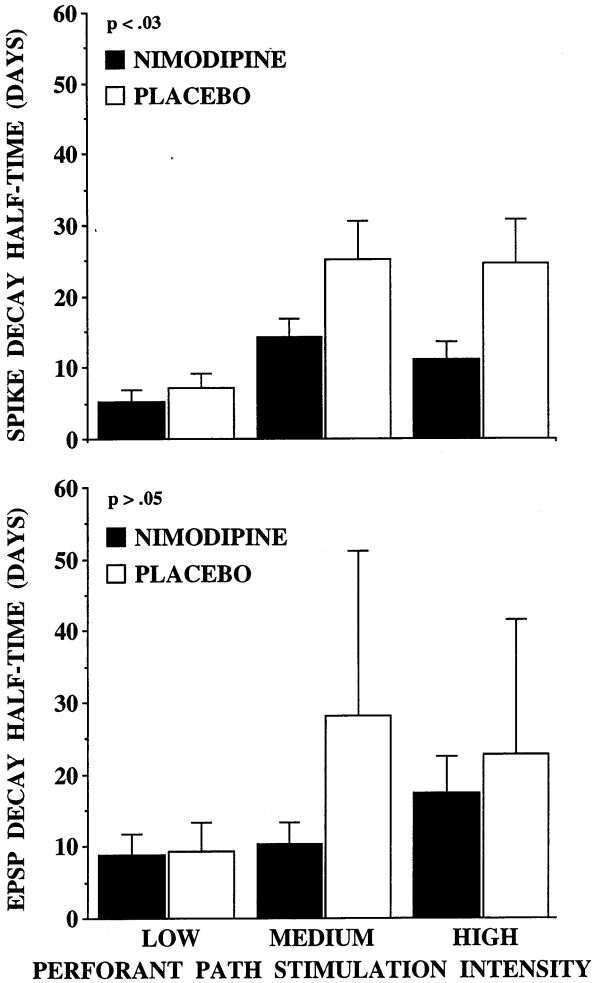

Fig. 4. Decay halftimes for the population spike and population EPSP at each of the three I/O intensities. The nimodipine treated group had significantly ($P < 0.03$) faster decay of the population spike than did the placebo group. There were no significant group differences in decay of the EPSP.

tered nimodipine than in rats administered placebo ($F(1,17) = 5.18$; $P < 0.03$). Although there were no significant differences between the two groups in EPSP decay rates, there was a trend towards faster decay of LTP in animals administered nimodipine.

3.3. Behavioural-electrophysiological correlations

As noted earlier, 19 animals had acceptable electrophysiological responses. For these animals, correlations between the trials to acquisition and the magnitude of LTP for both the population spike or EPSP were calculated. The correlations were not significant.

Correlation coefficients were also calculated between the decay constants and the number of trials required to reach the retention criterion. Only six of the 13 animals that completed the extended retention training had acceptable data for population spike analysis (if the population spike amplitude dropped below the pretetanus amplitude, decay constants were not calculated). For these six animals, there were no significant correlations between trials to the retention criterion and decay of the population spike. There were only two animals with acceptable data for both trials to the retention criterion and EPSP decay rates. Consequently, no correlation coefficients were calculated for these measures.

4. Discussion

The present study investigated the effect of chronic nimodipine administration on the acquisition and retention of spatial reference memory. In addition, the effect of nimodipine on the induction, magnitude and duration of LTP were examined. Comparison of the behavioural and electrophysiological effects of nimodipine administration allowed further examination of the proposed relationship between LTP and learning and memory.

4.1. Effect of nimodipine on spatial learning

Nimodipine improved both the acquisition and the retention of spatial reference memory as measured on the Barnes circular platform task. These results are in contrast to the lack of effect of nimodipine administration on acquisition of a water maze task [20,21]. Although both the water maze and circular platform tasks place demands on reference memory, the latter, with its 18 possible tunnel locations, may place stronger demands on mnemonic processes. Consequently, nimodipine-induced effects may be more apparent on the circular platform task. The present findings, together with the finding that nimodipine administration facilitates acquisition of an 8-arm working memory task [19], suggest that nimodipine administration has beneficial effects on spatial learning and memory regardless of the memory requirements (i.e. working or reference memory).

4.2. Effect of nimodipine on LTP

Nimodipine did not have a significant effect on the level of granule cell excitability prior to tetanus. Specifically, the baseline population spike and EPSP amplitudes were not altered in the presence of nimodipine. Previous studies [22,29] reported that similar doses of nimodipine increased hippocampal CA1 excitability. Hippocampal CA1 cells, however, have a higher concentration of L-type calcium channels than the dentate gyrus [23] and therefore may be more sensitive to L-type calcium channel antagonists.

In the present experiments, nimodipine administration also did not have a significant effect on the threshold intensity required for LTP induction or on

the magnitude of LTP. In contrast, Christie et al. [7] found a nimodipine-induced decrease in LTP threshold and an increase in the magnitude of LTP. It is possible that different tetanus protocols could account for the difference between the two studies. Christie et al. [7] utilized theta-burst stimulation to induce LTP and previous studies have demonstrated drug effects on LTP that were specific to the use of theta-burst stimulation [8].

The present results indicate that nimodipine administration did not alter the rate of decay of LTP of the EPSP but did increase the decay rate of LTP of the population spike. The maintenance of LTP in the first few hours post-tetanus may require presynaptic mechanisms (e.g. increased neurotransmitter release) [5] while later components may rely on postsynaptic mechanisms [9]. Postsynaptically, it has been found that there is an increase in intracellular calcium following LTP, not only near the activated synapse, but also near the cell body of the post-synaptic neuron [24]. Since L-type calcium channels are predominantly localized postsynaptically near the cell soma [30], the population spike results are in support of a postsynaptic component to the maintenance of LTP. Although EPSP decay was not significantly affected by nimodipine, Fig. 4b illustrates a trend similar to the significant effect of nimodipine on the decay of LTP of the population spike. As noted earlier, due to computer failure, EPSP data from seven animals were lost. It is possible that if the number of subjects for the EPSP analyses were increased, the observed trends would reach significance.

4.3. Lack of relationship between nimodipine's effect on spatial learning and LTP

Although LTP is considered a strong candidate neural mechanism for learning and memory, definitive-proof is lacking [11,12,14,28]. In the present study, the correlation between acquisition of the circular platform task and LTP magnitude was not significant. The correlation between retention performance and LTP durability for the population spike also was not significant. However, the latter correlation was based on only six animals, and only on the decay of the population spike. While it is possible that a significant relationship would occur if the number of animals were increased, or if it were calculated with decay data for LTP of the EPSP, the reported nonsignificant relationship was directly opposed to that expected if similar mechanisms were involved in both LTP and memory maintenance [2,4]. Instead of a positive relationship between the retention of memory and the maintenance of LTP, nimodipine improved the strength of memory and attenuated the durability of LTP. The lack of a simple one-to-one correspondence between tetanus-induced LTP magnitude/duration and learning/memory is consistent with numerous other findings [3,6,25,26]. Whether a relationship between spatial learning/memory and theta frequency-induced LTP exists is not known.

5. Summary

In conclusion, the present study confirms that nimodipine administration exerts beneficial effects on some component of the spatial memory system in young, neurologically intact animals. The results are also supportive of the theory that a post-synaptic, non-NMDA, calcium ion influx is involved in the maintenance of LTP. However, the same dosage of nimodipine that facilitated spatial learning and retention did not have a significant effect on hippocampal tetanus-induced LTP. Together, the results do not provide support for a similar mechanism playing a role in both spatial learning and tetanus-induced LTP.

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