Available online at www.sciencedirect.com
SCIENCE DIRECT®
Pharmacology, Biochemistry and Behavior 74 (2003) 269–278
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

The L-type calcium channel blocker nimodipine mitigates "learned helplessness" in rats☆

Samar Saade, Bernard W. Balleine, Thomas R. Minor*

Department of Psychology, University of California at Los Angeles, Los Angeles, CA 90095-1563, USA

Received 25 April 2002; received in revised form 8 July 2002; accepted 17 July 2002

Abstract

We assessed the effect of nimodipine, an L-type calcium channel blocker, on the escape deficit induced by prior exposure to inescapable shock in rats in four experiments. In Experiment 1, we injected rats at each of three time points (i.e., before shock exposure, after shock exposure, and before shuttle escape testing) with one of four doses of nimodipine (0, 0.5, 2.5, 5.0 mg/kg). The 5.0-mg/kg dose was most effective, acting to reduce shuttle escape latencies of inescapably shocked rats to a level comparable with nonshocked controls. No benefit occurred in Experiment 2, however, when nimodipine was administered at only one of the three time points used in the first experiment. Moreover, escape performance did not improve when rats received injections of nimodipine on the 2 days prior the experiment, and then one additional injection at one of the three time points identified above in Experiment 3. Finally, administration of nimodipine at two of the three time points did improve escape responding, but only when injected immediately prior both to shock exposure and the shuttle escape test.

Keywords: Helplessness; Stress; Nimodipine; L-type channel; Rats

1. Introduction

Acute experience with unsignaled, inescapable electric shock produces a profound deficit in adaptive behavior 24 h later. This *learned helplessness effect* (Maier and Seligman, 1976; Overmier and Seligman, 1967) or *distress syndrome* (Minor et al., 1991) has been demonstrated in many species and is used widely to model symptoms of major depression (Overmier and Hellhammer, 1988), severe anxiety (Ninan, 2001; Volpicelli et al., 1999), and the comorbidity between these disorders (Minor et al., 1994a,b, 2001; Weiss and Simson, 1985). Behavioral impairment in this paradigm is related to the induction and prolonged maintenance of intense fear during initial exposure to the uncontrollable stressor (Drugan et al., 1994; Jackson and Minor, 1988; Mineka et al., 1984; Minor, 1990; Minor et al., 1991; Weiss et al., 1981). The exact sequelae by which the initial emotional reaction translates into performance deficits 24 h later is not well understood. However, the problem appears to be, in part, a nonassociative consequence of prolonged and excessive activation of the neural substrates of fear.

Excessive excitation of brain neurons can challenge intracellular calcium homeostasis (Choi, 1988; Freund et al., 1992) and such ionic disequilibrium is thought to contribute to emotional disturbances (Blair et al., 2001; Emrich et al., 1993; Gareri et al., 2000). Serum calcium concentrations often are elevated in major depression (Dubovsky and Franks, 1983) and pharmacological regulation of calcium might prove to be a beneficial adjunct to traditional drug treatment (Suzuki et al., 2001; Taragano et al., 2001). In animal studies, increasing blood calcium concentrations via chronic oral administration in water impairs escape learning in a manner that is highly reminiscent of the learned helplessness effect in rats (Trulson et al., 1986). Conversely, pharmacological blockade of L-type calcium channels mitigates behavioral depression in rats, as measured by immobilization in the forced swim task (Czyrak et al., 1992; Tazi et al., 1992).

The present study examined the potential benefit of regulating calcium currents with the dihydropyridine nimodipine in the learned helplessness paradigm. Nimodipine is a potent antagonist of calcium influx via L-type calcium channels in both neurons and the brain vasculature. In

☆ This research was submitted in partial fulfillment of the requirement for a Master's Degree in Psychology at UCLA by Samar Saade.

* Corresponding author. Tel.: +1-310-825-3611; fax: +1-310-206-5895.
E-mail address: minor@psych.ucla.edu (T.R. Minor).

0091-3057/02/$ – see front matter © 2002 Elsevier Science Inc. All rights reserved.
PII: S0091-3057(02)00957-7

addition to the general evidence linking calcium dysregulation with emotional distress, the specific contribution of L-type channel activation to learning/memory process in fear conditioning (Blair et al., 2001), as well as its role in regulating blood flow and metabolic homeostasis in the brain microvascular bed (Catterall and Striessning, 1992; Goligorsky et al., 1995), suggest that nimodipine might be particularly effective in alleviating distress and helplessness in inescapably shocked rats. As discussed in detail later, nimodipine treatment at several time points in the helplessness procedure presumably could alter the sequelae that converge 24 h poststress to disrupt escape performance and other measures of adaptive behavior in inescapably shocked rats.

We assessed nimodipine's efficacy at alleviating helplessness in four parametric experiments, each of which focused on a different aspect of the drug treatment regimen. In each experiment, rats were exposed to a series of inescapable electric shocks or simple restraint in tubes and then tested for escape performance 24 h later in a shuttle box (Maier et al., 1973). We assessed three components of the drug treatment regimen to any beneficial action of nimodipine. First, although studies of nimodipine's action in a forced swim test provide a potential range of drug doses for comparison, we were unsure whether the available observations transcend models of depression. Thus, we assessed several doses of nimodipine for a beneficial action. Second, a single injection of nimodipine, or other types of L-type channel blockers, is ineffective at alleviating immobility in a forced swim test for behavioral depression (Biala, 1998; Czyrak et al., 1990; Czyrak, 1993; Tazi et al., 1992). These data suggest that some drug sensitization may be necessary before nimodipine exerts a therapeutic effect. Thus, we assessed the number of drug treatments required to improved escape performance in inescapably shocked rats. Finally, three time points in the helplessness procedure are known to be sensitive to experimental interventions (immediately before stress pretreatment, immediately after pretreatment, and immediately before testing). Thus, we determined whether nimodipine was particularly effective at any of these time points both singly and in combination.

2. Experiment 1

We assessed the effects of four different doses of nimodipine, with each dose administered three times during the course of the experiment to a different group of rats. Rats received an intraperitoneal injection of one of four doses of nimodipine (0, 0.5, 2.5, 5.0 mg/kg) both immediately before and immediately after the session in which they were exposed either to inescapable tail shock (IS) or simple restraint (R) in a complete factorial design. Rats in each of the shocked (IS-0, IS-0.5, IS-2.5, IS-5.0) and the restrained groups (R-0, R-0.5, R-2.5, R-5.0) conditions received a third injection of the same dose of nimodipine 24 h after stress treatment. All groups were tested for shuttle escape performance 20 min later.

By injecting rats prior to stress exposure, immediately poststress and shortly before escape testing, we treated animals at all significant time points at which other types of manipulations have been found to be effective. Moreover, the choice of these time points was based on empirical evidence of effect drug interventions in prior research, rather than on any special insight into the processes that are impacted by drug treatment at any given point in time. Thus, by injecting nimodipine at all three time points, we maximized the likelihood of observing a beneficial effect of nimodipine. Thus, if the detrimental effects of inescapable shock on later escape performance are, at least in part, produced by calcium influx via L-type channels, then test escape performance in inescapably shocked rats should improve in a dose-dependent manner relative to restrained controls.

2.1. Method

2.1.1. Subjects and apparatus

Sixty-four male Sprague–Dawley albino rats (285–310 g) from a breeding colony in the Psychology Department at the University of California at Los Angeles (UCLA) were housed in individual cages with free access to food and water in a room maintained on a 12:12-h light–dark cycle. Experimentation occurred in the light portion of the day/night cycle. All research works described herein received prior approval from the Institutional Animal Care and Use Committee.

Pretreatment occurred in clear Plexiglas restraining tubes, measuring 23 cm in length and 6 cm in diameter. Adjustable front walls prevented rats from moving forward in the tubes. A rat's tail extended through the rear door of each tube and was taped to a plastic rod. Unscrambled shock was delivered from a constant current generator (Model 82400; Lafayette Instruments, Lafayette, IN) to electrodes clamped to a rat's tail. Contact between the electrodes and the rat's tail was enhanced with electrode paste. Each tube was housed in a sound-attenuating chamber containing an exhaust fan that masked extraneous noise. Illumination was provided by a 7-W house light located in the center of the rear wall of the attenuating chamber.

Escape testing took place in three identical ($45 \times 20 \times 20$ cm) shuttle boxes (BRS-LVE model 146-40). Each shuttle box was divided into two identical compartments by a metal barrier that had an 8×7 cm center opening flush with the grid floor. The floor consisted of 2-mm-diameter stainless steel rods spaced 1.1 cm apart center to center. Scrambled shock was delivered to the grid floor from one of three Grason-Stadler (Series 700; West Concord, MA) shock generators. The floor was pivoted in the center and a response was recorded when a microswitch was activated by a floor depression in either compartment. Two 6-W lamps located in the center of the interior end wall of each compartment provided constant illumination. Each shuttle box was housed in a sound-attenuated chamber containing

an exhaust fan that masked extraneous noise. Experimental events were programmed and data were recorded by microcomputers.

2.1.2. Procedure: stress treatments and behavioral testing

Rats were assigned randomly to one of eight groups of eight rats each. Groups were created by the factorial combination of two stress treatment conditions (shock or restraint) and four drug doses of nimodipine (0, 0.5, 2.5, or 5.0 mg/kg). Four groups were restrained in tubes and exposed to 100 variable duration (mean = 8.0 s; range = 3–15 s) inescapable tailshocks (1.0 mA), with an average intershock interval of 60 s (range = 20–150 s). The other four groups were restrained in tubes for the same amount of time (1.83 h) and received no shock.

Twenty-four hours later, all rats were tested for escape performance in the shuttle boxes. The test consisted of five trials during which gridshock (0.6 mA) was terminated by a single shuttle response (FR-1 trials), followed by 25 trials during which the rat had to cross from one side of the shuttle box to the other and then return to terminate shock (FR-2 trials). Shock terminated automatically if the appropriate response was not made within 40 s of shock onset. Both trial types were presented on a variable time 60-s schedule (range = 20–230 s). A 3-min interval intervened between FR-1 and FR-2 trial types in order to maximize performance deficits in inescapably shocked rats (cf., Minor and LoLordo, 1984).

2.1.3. Drug treatment

Nimodipine was dissolved in a vehicle consisting of 20% ethyl alcohol, 60% propylene glycol, and 20% water at one of five concentrations (0, 0.5, 2.5, and 5.0 mg/ml). All injections occurred at a volume of 1 ml/kg. Each preshocked (IS-0, IS-0.5, IS-2.5, and IS-5.0) and restrained group (R-0, R-0.5, R-2.5, R-5.0) received one injection of one of these doses immediately prior to stress pretreatment, immediately after stress pretreatment, and approximately 24 h later (20 min prior to escape testing).

2.2. Results and discussion

2.2.1. FR-1 trials

Although escape latencies on the initial FR-1 trials are usually not affected by pretreatment stress or other experimental variables, the inescapably shocked group treated with the low dose of nimodipine (Group IS-0.5) showed a small but statistically reliable elevation in FR-1 latencies relative to shocked and restrained vehicle controls (Groups IS-0 and R-0), as well as all other nimodipine-treated groups [$F(1,62) = 4.76$, $P < .05$] and Newman–Keuls contrasts ($\alpha = .05$). Performance in all other groups did not differ statistically.

2.2.2. FR-2 trials

Mean FR-2 escape latencies for each inescapably shocked and restrained group are shown in Fig. 1. The

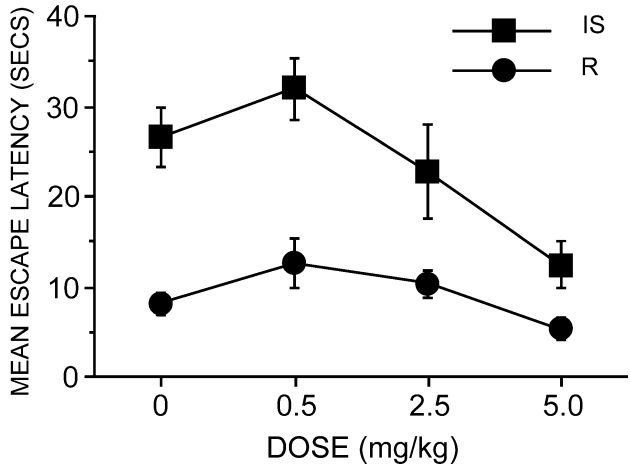

Fig. 1. Mean (±S.E.) FR-2 shuttle escape latencies for the inescapably shocked and restrained groups in Experiment 1. Rats received intraperitoneal injections of nimodipine or vehicle before exposure to inescapable shock, after exposure to inescapable shock, and before shuttle escape testing.

difference in response latencies between Groups IS-0 and R-0 establishes the boundaries of the helplessness effect. The detrimental effect of inescapable shock generally was reduced by nimodipine treatment in a dose-dependent manner. Although the 0.5-mg/kg nimodipine dose produced a slight increase in behavioral impairment in Group IS-0.5 relative to Group IS-0, performance generally improved from this level in the moderate (Group IS-2.5) and high (Group IS-5.0) doses of the drug. Indeed, performance in Group IS-5.0 appeared similar to that in the restrained control groups (R-0, R-0.5, R-2.5, and R-5.0), which were not differentially affected by drug treatment.

A mixed-design ANOVA (Stress × Drug Dose) conducted on FR-2 escape latencies yielded significant main effects of pretreatment Stress Condition [$F(1,312) = 240.26$, $P < .001$] and Drug Dose [$F(3,312) = 9.16$, $P < .001$]. The interaction between factors was not statistically significant. Newman–Keuls post hoc contrasts ($\alpha = .05$) found no difference among Groups R-0, R-0.5, R-2.5 and R-5.0, nor between Group IS-5.0 and any of these groups. Groups IS-0, IS-0.5, and IS-2.5 showed elevated latencies relative to all other groups. Unexpectedly, Group IS-0.5 differed from both Groups IS-0 and IS-2.5, although the latter two did not differ from each other.

The present data provide evidence that nimodipine can alleviate behavioral impairment in helpless rats, presumably by blocking Ca^{2+} influx via L-type channels. Escape latencies improved in inescapably shocked rats injected at three time points with moderate (2.5 mg/kg) nimodipine, with the high dose (5.0 mg/kg) completely eliminating any evidence of a deficit. Nevertheless, it must be conceded that the low dose of nimodipine increased the escape deficit in Group IS-0.5, an effect evident on both FR-1 and FR-2

trials. This outcome complicates any simple interpretation of the data.

These opposing effects of the high and low doses of nimodipine can be reconciled, however, if it is assumed that nimodipine has multiple (but opposing) mechanisms of action, which are differentially expressed in a dose-dependent manner. Indeed, nimodipine not only functions as a vasodilator and general antagonist of L-type channels, but also acts as a modest inhibitor of extracellular adenosine reuptake transport at low doses of the drug (Deckert and Gleiter, 1990).

We have argued that the transition from an initial state of anxiety and agitation, which is shown by inescapably shocked rats during the initial moments of escape testing, to a state of conservation withdrawal (or helplessness) is mediated by an increase in adenosine-induced inhibition of neuronal activity (Minor et al., 1994a,b; Woodson et al., 1998). The transition is blocked by pretest injection adenosine receptor antagonists, mimicked by pretest treatment of nonstressed rats with an adenosine receptor agonist (Minor et al., 1994b), or a compound that blocks the degradation of extracellular adenosine (Woodson et al., 1998). Most relevant to the present argument is the finding that the effect of inescapable shock on test performance is mimicked by pretest treatment of restrained rats with substances that block adenosine reuptake transport (Woodson et al., 1998).

Thus, nimodipine's properties as a nucleoside transport blocker should functionally increase the effects of extracellular adenosine. Importantly, an adenosine-enhancing effect of nimodipine would be expected only when a substantial concentration of adenosine is released into the synaptic cleft. If the low dose of nimodipine had little or no ability to ameliorate the effect of inescapable shock—viz., the ability to increase extracellular adenosine at the time of testing—then the drug's properties as an adenosine uptake inhibitor should have enhanced the escape deficit in Group IS-2.5 relative to Group IS-0, without substantially affecting performance in Group R-2.5. Nimodipine's property as a nucleoside transport blocker would become moot if the moderate and high drug doses progressively mitigated the ability of inescapable shock to provoke the release of extracellular adenosine at the time of testing. Test escape performance in Groups IS-2.5 and IS-5.0 would have improved relative to Groups IS-0 and IS-0.5.

3. Experiment 2

Experiment 1 established that an injection of 5.0 mg/kg nimodipine reduced the escape deficit induced by inescapable shock when administered immediately before pretreatment, immediately after pretreatment, and 20 min before the shuttle escape test. Experiment 2 determined whether a single injection of nimodipine at one of these three time points was effective. Three groups of rats received an injection of nimodipine (5.0 mg/kg ip) or vehicle either before exposure to inescapable shock, after exposure to inescapable shock, or 20 min before shuttle escape testing. Three additional groups, which were restrained in tubes during pretreatment, received similar injection conditions. To control for injection cues and maintain consistency with the procedure in Experiment 1, rats were injected with vehicle at the two time points where they were not injected with nimodipine. Thus, two groups—one pretreated with shock (IS) and one pretreated with restraint (R)—received vehicle (V) at all three time points (IS-VVV and R-VVV), two groups received nimodipine (N) prior to pretreatment (IS-NVV and R-NVV), two groups received nimodipine following pretreatment (IS-VNV and R-VNV), and two groups received nimodipine prior to shuttle escape test (IS-VVN and R-VVN).

3.1. Method

3.1.1. Subjects and apparatus

Sixty-four experimentally naive, male albino rats, weighing between 285 and 310 g, were obtained from the UCLA breeding colony and housed as in Experiment 1. The apparatus was the same as that described in Experiment 1.

3.1.2. Procedure

Rats were assigned randomly to one of eight groups of eight rats each. We exposed four of these groups to 100 variable duration inescapable shocks as described in Experiment 1. The other four groups were restrained for the same period of time and received no shock. Twenty-four hours later, FR-1 and FR-2 shuttle escape performance of all animals was assessed in the shuttle boxes using exactly the same procedures described in Experiment 1.

3.1.3. Drug treatment

All animals received three intraperitoneal injections. Rats in one shocked (IS) and one restrained (R) group received three injections of vehicle (1 ml/kg), one immediately before pretreatment, one immediately after pretreatment, and one 20 min prior to the shuttle escape test (IS-VVV and R-VVV). These groups served to set the boundaries of the standard learned helplessness effect. The remaining groups received two injections of vehicle and one injection of 5.0 mg/kg nimodipine (dissolved in vehicle to a concentration of 5.0 mg/ml). Two groups (one shocked and one restrained) were injected with nimodipine immediately prior to pretreatment and with vehicle immediately after pretreatment and 20 min prior to the test (IS-NVV and R-NVV). Two other groups (IS-VNV and R-VNV) were injected with nimodipine immediately after pretreatment, and with vehicle immediately prior to pretreatment and 20 min prior to the test. Two groups (IS-VVN and R-VVN) were injected with nimodipine 20 min prior to escape testing, and with vehicle immediately prior to and immediately after pretreatment.

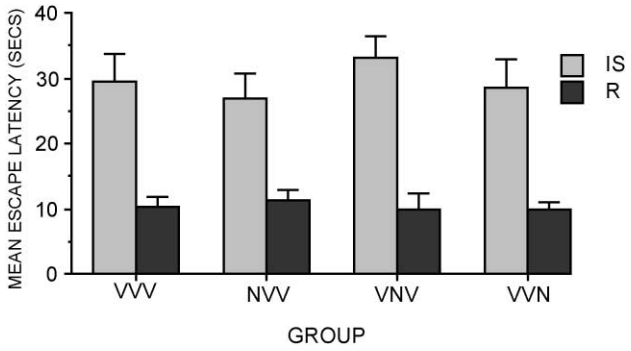

Fig. 2. Mean (±S.E.) FR-2 shuttle escape latencies for the inescapably shocked and restrained groups in Experiment 2. Rats were given vehicle (V) or one acute injection of nimodipine (N: 5.0 mg/kg) either before exposure to inescapable shock, following exposure to inescapable shock, or before shuttle escape testing. Vehicle was administered at the other two time points.

3.2. Results and discussion

Escape latencies did not differ among groups during FR-1 trials ($F < 1$). Mean FR-2 shuttle escape latencies in each of group are shown in Fig. 2. Time of drug treatment had no effect on the FR-2 escape latencies in either the IS or R group. Indeed, performance was determined solely by pretreatment stress condition, with the escape latencies of all three groups exposed to inescapable shock being substantially longer than those groups exposed to restraint.

A two-factor ANOVA (Stress Condition × Injection Time) of mean FR-2 latencies yielded a significant main effects of Stress Condition [$F(1,234) = 304.89$, $P < .001$], but neither a significant main effect of injection time nor an interaction between factors ($Fs < 1$).

These data clearly establish that a single 5.0 mg/kg injection of nimodipine is inadequate to mitigate the effects of inescapable shock on later escape performance, regardless of the time of the injection. FR-2 escape latencies in the nimodipine-treated inescapably shocked groups did not differ from the vehicle-treated shocked group.

4. Experiment 3

One potential explanation for the failure to see any benefit to a single injection of nimodipine in the previous experiment is that some sensitization to the drug occurs when it is administered repeatedly (e.g., as in Experiment 1). Indeed, sensitization does appear to be one of the characteristics of repeated treatment with nimodipine (Ossowska et al., 1994). In Experiment 3, we replicated exactly the procedure used in Experiment 2 except that, on each of 2 days immediately preceding pretreatment, rats were given an injection of nimodipine (5.0 mg/kg). Thus, if the failure to generate an effect of a single injection of nimodipine in Experiment 2 was due to a failure to induce comparable sensitization to that occurring with the three injections in Experiment 1, then giving two additional injections prior to pretreatment in Experiment 3 should overcome this problem.

In Experiment 3, therefore, six groups (three shock and three restraint-pretreated) were injected with nimodipine at one of three time points, either immediately before pretreatment (Groups IS-NVV and R-NVV), immediately after pretreatment (Groups IS-VNV and R-VNV), or 20 min prior to the shuttle escape test (Groups IS-VVN and R-VVN) as in Experiment 2. In addition, all animals in these groups were given an injection of nimodipine on each of 2 days prior to stress pretreatment. As in Experiment 2, injections of vehicle were given at the time points when nimodipine was not administered.

Although differences in sensitization provides a reasonable explanation for the differential effects of nimodipine observed in Experiments 1 and 2, we were troubled by the alternative possibility that the effect of nimodipine observed in Experiment 1 was due to chance and that the drug in reality had no effect on escape performance, as observed in Experiment 2. Given this possibility, we decided to make one further change to the procedures of Experiment 2. Instead of using two pretreatment vehicle control groups, injected twice with nimodipine and then with vehicle at all three time points, we decided to incorporate two groups given treatment similar to Groups IS-5.0 and R-5.0 in Experiment 1. These groups were given injections of vehicle on the 2 days prior to pretreatment and were then injected with nimodipine at all three time points, i.e., immediately before and immediately after pretreatment as well as 20 min prior to the test (Groups IS-NNN and R-NNN).

4.1. Method

4.1.1. Subjects and apparatus

Sixty-four male albino rats, weighing 285 g–310 g, were obtained from the UCLA breeding colony and housed as described in Experiment 1. The apparatus used was the same as in Experiment 1.

4.1.2. Procedure

Rats were assigned randomly to one of eight groups of eight rats each. Four groups were exposed to 100 variable duration inescapable shock as described in Experiment 1. The other four groups were simply restrained for the same period of time and received no shock. Twenty-four hours later, all groups were tested for escape performance in the shuttle boxes as described in Experiment 1.

4.1.3. Drug treatment

Six groups (three inescapably shocked and three restrained groups) received an injection of nimodipine (5.0 mg/kg ip) on each of the 2 days prior to stress pretreatment. All groups then received three injections—two injections of

vehicle and one injection of nimodipine—such that nimodipine was administered either (a) before pretreatment (i.e., IS-NVV and R-NVV), (b) following pretreatment (i.e., IS-VNV and R-VNV), or (c) 20 min before shuttle escape test (i.e., IS-VVN and R-VVN). The remaining two groups received vehicle for the 2 days prior to stress pretreatment and then an injection of nimodipine at each of the three time points (i.e., IS-NNN and R-NNN). The vehicle was the same as in Experiment 1.

4.2. Results and discussion

Escape latencies did not differ among groups during FR-1 trials [$F(1,62) = 2.55$, $P > .05$]. Mean FR-2 escape latencies for each of the groups used in Experiment 3 are presented in Fig. 3. Nimodipine administered at only one of the three time points had no effect on FR-2 shuttle escape latency, even when priming injections were given on each of the 2 days prior to the start of pretreatment. Nevertheless, as we observed in Experiment 1, when nimodipine was administered at all three time points, shuttle escape latency in inescapably shocked rats (Group IS-NNN) did not differ from the restraint-pretreated control (Group R-NNN). As such, the three injections of nimodipine were effective in mitigating the effects of inescapable shock but only when they were given before or immediately after a shock session.

A mixed-design ANOVA (Stress Condition × Injection Time) of mean FR-2 escape latencies yielded both a main effect of Stress Condition [$F(1,312) = 158.3$, $P < .001$] and of Injection Time [$F(3,312) = 21.9$, $P < .001$], as well as a significant interaction between these factors [$F(3,312) = 27.41$, $P < .001$]. Newman–Keuls pairwise comparisons ($\alpha = .05$) conducted on the mean FR-2 latencies indicated that the escape latencies in Groups IS-NVV, IS-VNV, and IS-VVN were significantly longer than all other groups. The restrained groups (R-NVV, R-VNV, R-VVN, and R-NNN) did not differ. Group IS-NNN did not differ from any of the restrained groups but differed reliably from each of the remaining inescapable shock groups.

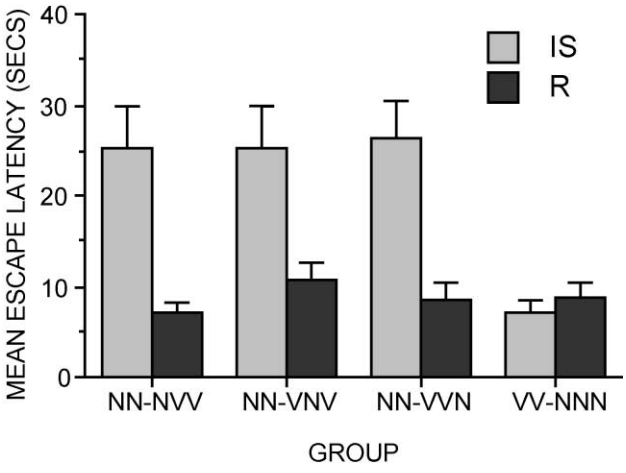

Fig. 3. Mean (±S.E.) FR-2 shuttle escape latencies for inescapably shocked and restrained groups in Experiment 3. Rats were given three injections of nimodipine (5.0 mg/kg) such that two of the injections were administered 2 days prior to the start of the experiment and the third injection was given either before exposure to inescapable shock, following exposure to inescapable shock, or prior to shuttle escape testing.

Experiment 3 replicated the results of both Experiments 1 and 2 using a procedure in which all the rats received three injections of nimodipine. Thus, three injections of nimodipine were effective in mitigating the effects of inescapable shock but only when they were given before or immediately after a shock session. When two injections were given prior to pretreatment and only one injection was given at one of the three time points in each of the remaining groups, a deficit in escape performance was observed for inescapably shocked rats. Thus, contrary to the sensitization account devised to explain the results of Experiment 2, administering two priming injections of nimodipine failed to reveal an effect of the third injection.

5. Experiment 4

The previous experiments found that nimodipine does not reduce the escape deficits induced by inescapable shock when injected only once during the experimental sessions. The drug is effective, however, when injected at three time points during these sessions. Thus, although it is clear that an injection of nimodipine at all three times points is *sufficient* to prevent the debilitating effects of inescapable shock, it is unclear whether three injections are *necessary* for this effect.

This question was addressed in Experiment 4 using five groups of rats. Because we were primarily interested in assessing the influence of nimodipine on rats exposed to inescapable shock, all five groups were given shock pretreatment followed 24 h later by the shuttle escape test. Three of the groups were given one injection of nimodipine (5.0 mg/kg ip) on the day prior to the pretreatment shock session followed by two further injections of nimodipine: Group NNV was injected both immediately before and immediately after exposure to inescapable shock; Group NVN was injected immediately before exposure to inescapable shock and 20 min before the shuttle escape test; and Group VNN was injected immediately after exposure to inescapable shock and 20 min before the shuttle escape test. An injection of vehicle was given at time points when nimodipine was not scheduled. Finally, the two remaining groups, Groups NNN and VVV, were included to provide boundary conditions against which to assess the effects of the other treatments. Both groups received an injection of vehicle on the day prior to the pretreatment shock session and then three further injections: Group NNN was given an injection of nimodipine at each of the three time points, whereas Group VVV received injections of vehicle only.

5.1. Method

5.1.1. Subjects and apparatus

Forty male albino rats, weighing between 285 and 310 g, were obtained from the UCLA breeding colony and housed as described in Experiment 1. The apparatus used was the same as that described in Experiment 1.

5.1.2. Procedure

Rats were assigned randomly to one of five groups ($n = 8$). All five groups were exposed to 100 variable duration inescapable shocks as described in Experiment 1 and then, 24 h later, were given a shuttle escape test, again as described in Experiment 1.

5.1.3. Drug treatment

Three groups received an injection of nimodipine (5.0 mg/kg) 1 day prior to the start of the pretreatment phase and two further injections during the course of the experiment either: (a) immediately before pretreatment and immediately after pretreatment (NNV); (b) immediately before pretreatment and 20 min before the shuttle escape test (NVN); or (c) immediately after pretreatment and 20 min before shuttle escape test (VNN). The remaining two groups received a vehicle injection 1 day before the pretreatment session and then either an injection of nimodipine at each of the three time points (NNN) or vehicle at all three time points (VVV). The vehicle was the same as in Experiment 1.

5.2. Results and discussion

Escape latencies did not differ among groups on the FR-1 trials ($F < 1$). Mean escape latencies on the FR-2 trials in each of the five groups are shown in Fig. 4. A clear benefit to FR-2 escape latencies was evident in Group NNN relative to Group VVV. Furthermore, a similar reduction in latency was observed in Group NVN, which received injections of nimodipine before the pretreatment and before testing. No reduction in latencies occurred in Group NNV or VNN.

A mixed-design ANOVA (Group × Trial Block) conducted on FR-2 escape latencies yielded a significant main effect of Group [$F(5,234) = 49.25$, $P < .001$]. Newman–Keuls pairwise comparisons ($\alpha = 0.05$) of mean FR-2 escape latencies indicated that Groups NNN and NVN did not differ. Escape latencies in both of these groups were significantly reduced compared with Groups VVV, VNN, and NNV, which did not differ from one another.

These data indicate that the pattern of injections matters. Although injecting inescapably shocked rat prior to stress, immediately poststress, and before testing is sufficient to prevent the helplessness effect, it is not necessary to give an injection of nimodipine at all three of these time points. A comparison of Groups NVN, NNV, and VNN on escape responding clearly indicates that an injection of nimodipine post pretreatment did not contribute to the reduction in escape latency observed in Group NNN. Further, although

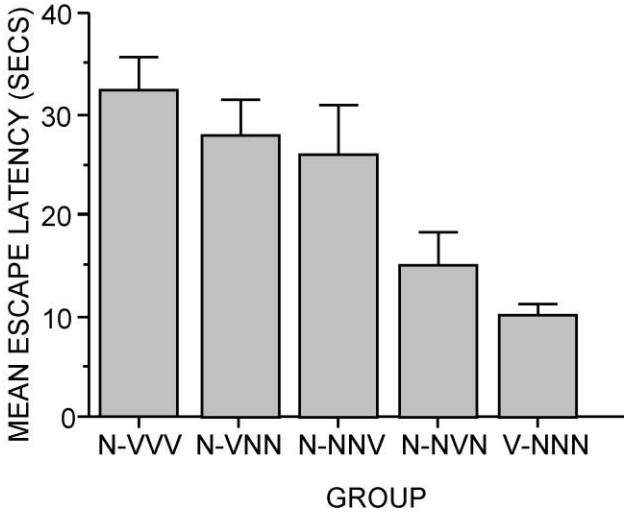

Fig. 4. Mean (±S.E.) FR-2 shuttle escape latencies for inescapable shocked groups in Experiment 4. One group received a nimodipine injection 24 h prior to pretreatment and then injections of vehicle immediately prior to pretreatment, immediately after pretreatment, and 24 h later, prior to shuttle escape testing. The other groups received three injections of nimodipine (5.0 mg/kg) such that one injection was given 24 h prior to start of experiment. The remaining two injections were given either before exposure to inescapable shock and following exposure to inescapable shock or before exposure to inescapable shock and before shuttle escape test, or following exposure to inescapable shock and before shuttle escape testing.

it is possible that the injection given after pretreatment acted to increase (rather than reduce) subsequent escape latencies, the very similar levels of performance observed in Groups NNN and NVN argue against this possibility. If an injection post pretreatment acted to increase escape latency, then the latencies of Group NNN should have been longer than those of Group NVN, but this was not observed. Instead, it seems reasonable to conclude that the injection of nimodipine given after pretreatment had no effect on subsequent escape performance. Thus, it appears that injections of nimodipine prior to stress pretreatment and prior to testing are necessary conditions for a beneficial effect of the drug.

6. General discussion

The present series of experiments assessed the ability of various treatment regimens with nimodipine, a dihydropyridine L-type calcium channel blocker, to ameliorate shuttle escape deficits resulting from earlier exposure to inescapable shock. Experiment 1 demonstrated that a high nimodipine dose (5 mg/kg), but not low (0.5 mg/kg) or intermediate doses (2.5 mg/kg), is effective in substantially improving test escape performance in inescapable shocked rats when drug injections occur prior to stress induction, immediately poststress, and prior to escape testing 24 h later. Nonetheless, a single injection of the high nimodipine dose failed to improve test performance in Experiment 2, regardless of whether we injected the drug prior to stress,

immediately poststress, or prior to testing. We also obtained a similar failure to improve test performance in Experiment 3, in which groups of rats received two nimodipine injections prior to the beginning of the experiment and then one additional injection at one of the three time points under study. Although injecting nimodipine at all three time points is sufficient to mitigate the effects of inescapable shock on later shuttle escape performance, the results of Experiment 4 suggest that all three injections are not necessary for such a benefit. We observed a similar degree of improvement in escape performance in Experiment 4 when we injected rats prior to pretreatment and prior to testing, but omitted the injection immediately poststress. No other combination of two injection times was effective. Overall, this pattern of results suggests that some process involving L-type Ca^{2+} channel activation is a necessary, but not a sufficient, condition for the behavioral impairment observed following inescapable shock.

These data are consistent with other observations that link distress and helplessness to changes in calcium homeostasis and L-type channel activation. Increasing blood Ca^{2+} concentrations via chronic oral administration in water produces deficits in escape learning, which lead the authors to speculate that high Ca^{2+} in brain contributes to the helplessness effect (Trulson et al., 1986). Conversely, preventing vasoconstriction via pharmacological blockade of L-type Ca^{2+} channels mitigates behavioral depression, as measured by immobilization in the forced swim test (Czyrak et al., 1992; Tazi et al., 1992).

One process that might be affected by nimodipine treatment in these stress experiments is the consolidation of fear conditioning. Certain elements of the pretreatment context are strongly associated with inescapable shock during the original stress session (Minor, 1990; Minor and LoLordo, 1984). Similar cues in the testing apparatus (e.g., stress odorants) greatly exaggerate the initial fear reaction to the situation 24 h later (Maier, 1990; Minor, 1990) and are necessary for the development of escape deficits. Removal of these strongly conditioned pretreatment cues from the testing apparatus eliminates deficits in escape performance in inescapably shocked rats. In this regard, nimodipine treatment may have disrupted the consolidation or transfer of fear conditioning in the present experiments. Activation of L-type Ca^{2+} channels is necessary for NMDA-independent long-term potentiation (LTP) (Grover and Teyler, 1990), a type of experience-dependent synaptic plasticity and a putative mechanism for learning and memory. LTP is induced in several pathways converging on and within the lateral nucleus of the amygdala during fear conditioning (e.g., Clugnet and LeDoux, 1990). These sites include thalamic nuclei that carry information concerning conditional stimuli to the lateral amygdala. The induction of LTP at these sites requires L-type channel activation (Weisskopf et al., 1999). The resulting influx of calcium presumably initiates a sequence of events that consolidate the memory trace in synaptic processes that afford later expression of the conditioned fear association (Blair et al., 2001). As such, blockade of these channels with nimodipine administration in the present experiments might have disrupted the consolidation of fear conditioning in inescapably shocked rats, thereby eliminating a necessary condition for behavioral impairment at the time of shuttle escape testing.

Nimodipine treatment also might alleviate symptoms of helplessness via a nonassociative process. For instance, vascular tone is controlled by inward movement of blood-borne Ca^{2+} ions through the voltage-gated L-type channels (Catterall and Striessning, 1992), which line the smooth muscle of the brain microvascular bed (Goligorsky et al., 1995). Under continuing high levels of activation, Ca^{2+} is taken up into the vasculature, causing the blood vessels to constrict, thus decreasing blood flow and the delivery of glucose and oxygen (Hogan and Hakim, 1992; Robertson and Robertson, 1996). This process can disrupt the essential coupling of regional blood flow to neuronal activation (Catterall and Striessning, 1992; Goligorsky et al., 1995; Lanier, 1999; Zager and Ames, 1988). Failure to achieve adequate dilation of these vesicles following excitatory transmission deprives the target neuron of metabolic substrates at a time of high-energy expenditure (Hogan and Hakim, 1992; Robertson and Robertson, 1996). Such conditions can challenge regional energy homeostasis and dramatically alter normal synaptic communication among neurons.

This type of process, with neural metabolic failure as a crucial endpoint, is now implicated in the emergence of behavioral depression in helpless rats by a variety of data (Minor and Hunter, 2002; Minor et al., 1994a,b, 2001; Woodson et al., 1998). Metabolic failure in brain is met with a substantial increase in adenosine signaling. The nucleotide is released from fatigued neurons and binds to specific extracellular purine receptors. The major effect of adenosine receptor activation is extraordinarily potent form of neural inhibition (Deckert and Gleiter, 1990; Meghji, 1991). The functional consequence of this cascade in the helplessness procedure is that brain neurons, most likely the ones that are mediating escape behavior (see Minor et al., 2001), are potently inhibited and no longer contribute to active attempts to cope with environmental demands. Escape deficits ensue. From this view, treatment with nimodipine may have alleviated symptoms of helplessness by reducing calcium-induced vasoconstriction in critical brain regions. An enhanced supply of metabolic substrates during periods of high neuronal activation during escape testing might have reduced the challenge to metabolic homeostasis and the resulting cascade of events that lead to adenosine-mediated escape deficits.

The present data do not discriminate between associative and nonassociative accounts of nimodipine's beneficial action. Indeed, the alternatives discussed above are not necessarily mutually exclusive. Considerably more research is needed to discriminate between the above alternatives and

other potential explanations. Nonetheless, these experiments indicate that the helplessness procedure should prove as a useful tool in identifying nimodipine's beneficial action. Moreover, these data are consistent with the suggestion that nimodipine may prove to be a useful adjunct in the pharmacological treatment of anxiety and depression (Biala, 1998; Emrich et al., 1993; Gareri et al., 2000; Ninan, 2001; Suzuki et al., 2001; Taragano et al., 2001).

Acknowledgements

The work was supported by grants from the University of California Academic Senate and the Norman Cousin's Center for Psychoneuroimmunology to T.R.M. and a grant from the National Institute of Health to B.B.

References

Biala G. Antidepressant-like properties of some serotonin receptor ligands and calcium channel antagonists measured with the forced swimming test in mice. Pol J Pharmacol 1998;50:117–24.

Blair HT, Schafe GE, Bauer EP, Rodrigues SM, LeDoux JE. Synaptic plasticity in the lateral amygdala: a cellular hypothesis of fear conditioning. Learn Mem 2001;8(5):229–42.

Catterall WA, Striessning J. Receptor sites for Ca^{++} channel antagonists. TIPS 1992;13:256–62.

Choi DW. Glutamate neurotoxicity and diseases of the nervous system. Neuron 1988;1:623–34.

Clugnet MC, LeDoux JE. Synaptic plasticity in fear conditioning circuits: induction of LTP in the lateral nucleus of the amygdala by stimulation of the medial geniculate body. J Neurosci 1990;10(8):2818–24 (Aug).

Czyrak A. The effect of chronic nifedipine and ECS in the forced swimming test in rats. Pol J Pharmacol 1993;45:191–5.

Czyrak A, Mogilnicka E, Siwanowicz J, Maj J. Some behavioral effects of repeated administration of calcium channel antagonists. Pharmacol Biochem Behav 1990;35:557–60.

Czyrak A, Rogoz Z, Skuza G, Zajaczkowski W, Maj J. Antidepressant activity of S-adenosyl-L-methionine in mice and rats. J Basic Clin Physiol Pharmacol 1992;3(1):1–17 (Jan–Mar).

Deckert J, Gleiter CH. Adenosinergic psychopharmaceuticals: just an extra cup of coffee? J Psychopharmacol 1990;4(4):183–7.

Drugan RC, Basile AS, Ha J-H, Ferland RJ. The protective effects of stress control may be mediated by increased brain levels of benzodiazepine receptor agonists. Brain Res 1994;661(1–2):127–36.

Dubovsky SL, Franks RD. Intracellular calcium ions in affective disorders: a review and an hypothesis. Biol Psychiatry 1983;18(7):781–97 (Jul).

Emrich HM, Dose M, Wolf R. The action of mood-stabilizers in affective disorders: an integrative view as a challenge. Neuropsychobiology 1993; 27(3):158–62.

Freund TF, Ylinen A, Miettinen R, Pitkanen A, Lahtinen H, Baimbridge KG, Riekkinen PJ. Pattern of neuronal death in the rat hippocampus after status epilepticus. Relationship to calcium binding protein content and ischemic vulnerability. Brain Res Bull 1992;28(1):27–38.

Gareri P, Falconi U, De Fazio P, De Sarro G. Conventional and new antidepressant drugs in the elderly. Prog Neurobiol 2000;61(4):353–96.

Goligorsky MS, Colflesh D, Gordienko D, Moore LC. Branching points of renal resistance arteries are enriched in L-type calcium channels and initiate vasoconstriction. Am J Physiol 1995;268(2 pt 2):251–7.

Grover LM, Teyler TJ. Two components of long-term potentiation induced by different patterns of afferent activation. Nature 1990;347:477–9.

Hogan MJ, Hakim AM. Reversibility of nimodipine binding to brain in transient cerebral ischemia. J Neurochem 1992;59(5):1745–51.

Jackson RL, Minor TR. The effects of signaling inescapable shock on later escape learning: implications for theories of coping and "learned helplessness". J Exp Psychol, Anim Behav Processes 1988;14:390–400.

Lanier WL. The prevention and treatment of cerebral ischemia. Can J Anaesthesiol 1999;46(5):46–56.

Maier SF. Diazepam modulation of stress-induced analgesia depends on the type of analgesia. Behav Neurosci 1990;104(2):339–47.

Maier SF, Seligman MEP. Learned helplessness: theory and evidence. J Exp Psychol Gen 1976;105:3–46.

Maier SF, Albin RW, Testa TJ. Failure to learn to escape in rats previously exposed to inescapable shock depends on the nature of the escape response. J Comp Physiol Psychol 1973;85:581–92.

Meghji P. Adenosine production and metabolism. In: Stone TW, editor. Adenosine in the Nervous System. San Diego: Academic Press; 1991. p. 25–39.

Mineka S, Cook M, Miller S. Fear conditioning with escapable and inescapable shock: effect of a feedback stimulus. J Exp Psychol, Anim Behav Processes 1984;10:307–24.

Minor TR. Conditioned fear and neophobia following inescapable shock. Anim Learn Behav 1990;18:212–26.

Minor T.R., Hunter A.M.. Learned helplessness research: sensitization and fatigue processes. Integr. Physiol. Behav. Sci. 2002;37(1):44–58.

Minor TR, LoLordo VM. Escape deficits following inescapable shock: the role of contextual odor. J Exp Psychol, Anim Behav Processes 1984;10: 168–81.

Minor TR, Dess NK, Overmier JB. Inverting the traditional view of "learned helplessness". In: Denny MR, editor. Fear, avoidance and phobias: a fundamental analysis. Hillsdale (NJ): Lawrence Erlbaum Associates; 1991. p. 87–133.

Minor TR, Chang W-C, Winslow JL. Stress and adenosine: I. Effect of methylxanthine and amphetamine stimulants on learned helplessness in rats. Behav Neurosci 1994a;108:254–64.

Minor TR, Winslow JL, Chang W-C. Stress and adenosine: II. Adenosine analogs mimic the effect of inescapable shock in shuttle-escape performance in rats. Behav Neurosci 1994b;108:265–76.

Minor TR, Rowe M, Job RFS, Ferguson EC. Escape deficits induced by inescapable shock and metabolic stress are reversed by adenosine receptor antagonists. Behav Neurosci 2001;120:203–12.

Ninan PT. Recent perspectives on the diagnosis and treatment of generalized anxiety disorder. Am J Manag Healthc 2001;7(11):367–76.

Ossowska G, Klenk-Majewska B, Lupina T. The effect of calcium channel antagonists on the aggressive behavior in chronically stressed rats. Pol J Pharmacol 1994;46:403–8.

Overmier JB, Hellhammer DH. The learned helplessness model of human depression. In: Simon P, Soubrie P, Widlocher D, editors. An inquiry into schizophrenia and depression. Animal models of psychiatric disorders, vol. 2. Basel, Switzerland: Karger; 1988. p. 177–202.

Overmier JB, Seligman MEP. Effects of inescapable shock on subsequent escape and avoidance learning. J Comp Physiol Psychol 1967; 63:28–33.

Robertson RM, Robertson D. Drugs used for the treatment of myocardial ischemia. In: Molinoff PB, Ruddon RW, editors. Goodman and Gilman's the pharmacological basis of therapeutics 9th ed. New York: McGraw-Hill; 1996. p. 759–79.

Suzuki K, Kusumi I, Sasaki Y, Koyama T. Serotonin-induced platelet intracellular calcium mobilization in various psychiatric disorders: is it specific to bipolar disorder? J Affect Disord 2001;64(2–3):291–6.

Taragano FE, Allegri R, Vicario A, Bagnatti P, Lyketsos CG. A double blind, randomized clinical trial assessing the efficacy and safety of augmenting standard antidepressant therapy with nimodipine in the treatment of 'vascular depression'. Int J Geriatr Psychiatry 2001;16(3): 254–60.

Tazi M, Farh M, Moumni M, Hakkou F. Potentiation of behavioral effects of a calcium channel antagonist, nifedipine, by ipsapirone. Behav Pharmacol 1992;3(3):269–73.

Trulson ME, Arasteh K, Ray DW. Effects of elevated calcium on learned helplessness and brain serotonin metabolism in rats. Pharmacol Biochem Behav 1986;24:445–8.

Volpicelli J, Balaraman G, Hahn J, Wallace H, Bux D. The role of uncontrollable trauma in the development of PTSD and alcohol addiction. Alcohol Res Health 1999;23(4):256–62.

Weiss JM, Simson PG. Neurochemical mechanisms underlying stress-induced depression. In: Fields T, McCabe P, Schneiderman N, editors. Stress and coping. Hillsdale (NJ): Lawrence Erlbaum Associates; 1985. p. 93–116.

Weiss JM, Goodman P, Losito BG, Corrigan S, Charry JM, Bailey WH. Behavioral depression produced by an uncontrollable stressor: relationship to norepinephrine, dopamine and serotonin in various regions of rat brain. Brain Res Rev 1981;3:167–205.

Weisskopf MG, Bauer EP, LeDoux JE. L-type voltage-gated calcium channels mediate NMDA-independent associative long-term potentiation at thalamic input synapses to the amygdala. J Neurosci 1999;19(23):10512–9.

Woodson JC, Minor TR, Job RFS. Inhibition of adenosine deaminase by erythro-9-(2-hydroxy-3-nonyl) adenine (EHNA) mimics the effect of inescapable shock on escape learning in rats. Behav Neurosci 1998;112(2):399–409.

Zager EL, Ames A. Reduction of cellular energy requirements. Screening for agents that may protect against CNS ischemia. J Neurosurg 1988;69(4):568–79.