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Behavioral and Analgesic Effects Induced by Administration of Nifedipine and Nimodipine

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MARTÍN, M. I., V. L. DEL VAL, M. I. COLADO, C. GOICOECHEA, AND M. J. ALFARO. *Behavioral and analgesic effects induced by administration of nifedipine and nimodipine.* PHARMACOL BIOCHEM BEHAV **55**(1) 93–98, 1996.—Evidence exists that calcium antagonists can have effects on neural function. The aim of this work is to analyze the effect of two dihydropyridines, nifedipine and nimodipine, administered for 11 days on the behavior and pain sensitivity of rats. Nociception was tested using the tail electric stimulation test, and behavior parameters using a holeboard. Our results show that chronic administration of nifedipine or nimodipine induces analgesia that can be evaluated by tail withdrawal. However, neither the vocalization nor the vocalization after discharge were modified, so the analgesia may be mediated by spinal mechanisms. Rats treated with nifedipine or nimodipine exhibited a dose-dependent tendency to avoid the center of the field without modification of other parameters, suggesting an increased emotivity in the rats. This conclusion is supported by the fact that anxiogenic or anxiolytic drugs modify the pattern of locomotion without significant changes in other parameters related with the motility. The results from this study suggest the view of a complex mechanism of action underlying nifedipine- and nimodipine-mediated behavioral effects.

Nifedipine Nimodipine Behavior Pain Analgesia Anxiety Holeboard

THE calcium antagonists are chemically heterogenous drugs that inhibit the uptake of calcium into cells through L-voltage–dependent channels. Calcium channels appear to exist in all neurons and provide a significant proportion of the activator calcium required for neurotransmitter release. Evidence exists that calcium antagonists can have effects on neural function (43) and binding studies carried out using dihydropyridine calcium antagonists showed that drugs, such as nimodipine, have high affinity for membrane sites in the brain.

Calcium antagonists have been reported to be effective in the treatment of cardiac arrhythmias and other cardiovascular pathologies (9,10). Several reports and some drug trials suggest that they are also effective in the treatment of affective disorders such as maniatic symptoms (15,16,19), depression (25,37), behavioral changes induced by acute or chronic opioid treatments (4,24,28), convulsions (13), and withdrawal symptoms and seizures in alcohol-dependent rats (27). The acute but not chronic administration of nimodipine modifies the nociceptive threshold in rats (14). These actions have been described after both systemic and intracerebroventricular administration. Nimodipine (23) and nifedipine (17) are able to pass the blood–brain barrier, although nimodipine, more lipophilic, reach higher levels in the central nervous system.

However, there are relatively few reports studying the behavioral effects of the chronic administration of calcium antagonists, even though these drugs are usually administered chronically.

The aim of the present work was to asses possible behavioral alterations induced by chronic treatments with two dihydropyridines, nifedipine and nimodipine. We decided to use two different approaches for this analysis. Thus, we studied both the different drives expressed by the spontaneous behavior of the animal and its reaction to an external aversive stimulus.

First, the possible modifications in spontaneous behavior were evaluated by using the holeboard test. This test allows separate measurement of direct exploration (head dipping) and locomotion (1,11,18,44,48). Furthermore, and in accordance with previous studies by other authors and our own group, external and internal ambulation were recorded as different parameters, given the characteristic thigmotaxis of the rat (1,11).

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Second, we used a nociceptive test to assess possible alterations in the reaction of the animals to an external (nociceptive) stimulus. We have recently reported the validity of the tail electric stimulation test as a suitable methodology for the study of different processes involved in nociceptive and antinociceptive mechanisms (39,47). In contrast with other tests commonly used, such as tail flick and paw pressure, the tail electric stimulation test allows the study of different pain reactions integrated at different levels within the central nervous system (12,26,33,41). This, in turn, may provide additional information about the affective/emotional component of pain (30) that may be useful in analyzing the effects of calcium antagonists chronically administered.

METHOD

Male Sprague–Dawley rats, weighing 200–250 g at the beginning of the experiment, were individually housed in clear plastic cages and maintained in a temperature- and light-controlled environment on a reversed light cycle (lights off 0700 h, lights on 1700 h) with free access to food and water. Each group was allowed a 7-day period for acclimatization to the animal room. Behavioral and analgesic tests were carried out in the same room. The influence of the isolation has not been considered because the comparison has been done between groups housed in the same conditions. Food (g/kg) and water (ml/kg) intake and body weight (% of increase) were monitored daily throughout the experimental period. The testing and data recording were performed by an observer who was unaware of the drug treatment in each experiment.

Treated animals were given subcutaneous implants of osmotic minipumps (Alzet 2002) under light ether anesthesia (14).

Animals were randomly allocated to the following groups: 1) control: untreated animals; 2) vehicle-treated group: implanted with Tween-80 (2%)- filled osmotic minipumps; 3, 4, and 5) nifedipine-treated groups: implanted with nifedipine-filled osmotic minipumps delivering 200, 400, or 600 μg/kg/day respectively; and 6, 7, 8) nimodipine-treated groups: implanted with nimodipine-filled osmotic minipumps delivering 200, 400, or 600 μg/kg/day. Each group was comprised of at least 12 rats at the beginning of the experiences.

Nociception was tested using the tail electric stimulation test. The animals were placed in horizontal aerated plastic cylinders of a proper diameter for each animal and their tails were carefully cleaned. Inescapable tail shocks were delivered through fuse clip electrodes taped to the base of the tail and augmented by electrode paste. The electrodes were separated by 1.5 cm from each other and connected to a stimulator (SH-92 Cibertec) delivering variable intensity pulses of 60 Hz of frequency, train duration 100 ms, and train interval 5 s. The initial intensity was 0.05 mA, and this was increased until a response was observed. When there was no responses, the test was stopped at an intensity of 4.71 mA to avoid damage to the tissues. The thresholds (mA) for the motor response (tail withdrawal), vocalization during stimulation (vocalization), and vocalization after cessation of the stimulus (vocalization after discharge) were evaluated for each rat. These responses are accepted as being integrated respectively at: spinal level, medulla oblongata, and diencephalon-rhinencephalon (12,30,39,41).

The nociceptive test was performed at three points: 3 days before the implantation of the osmotic pumps (day −3), to determine baseline latencies before any manipulation took place, and days 3 and 11 after the minipump implantation. These measurements were done to assess possible differences in short- and long-term effects.

In order to make the comparison between the different groups easier, the nociception was quantified using the following formula:

$$\frac{\text{Intensity measured on day (n)}}{\text{Intensity measured on day } -3} = \frac{I_n}{I_B}$$

I_B being the basal-intensity, registered on day −3, for every response in a given rat, and In the intensity registered on days 3 and 11 to induce the same response in the same animal (40).

Behavioral changes were studied using a holeboard. The holeboard-test was performed only once in each rat at day 10 of treatment. The holeboard consisted of a black box (60 × 60 × 35 cm) divided by lines into 36 squares (10 × 10 cm) and had 4 equally spaced holes in it with diameters of 3.8 cm. For an experimental session each animal was gently placed in a corner of the box and allowed to explore freely for 5 min. Then, the following behavioral elements were recorded during 5 min: 1) frequency and duration of head-dipping; 2) frequency of rearing (a rear was recorded when the rat stood on its hind legs away from or up against a wall, to sniff the air); and 3) frequency of line crossing. During this time the path of the rat in the field was recorded on a map; the number of crossings was counted taking into account two areas, the peripheral and central regions (1,18,44,48).

The chamber was thoroughly cleaned and dried between animals. Test sessions were conducted during the dark phase of the light/dark cycle; the only light was a red light bulb.

Analysis of Data

The data were tested for homogeneity of variance between groups and then one-way ANOVA (factor treatment) or two-way ANOVA for nociceptive tests (factor 1 treatment, factor 2 day) was performed. Post hoc comparisons by LSD test were carried out when ANOVA showed statistically significant differences. A level of probability < 0.05 was accepted as statistically significant.

RESULTS

None of the treated groups showed significant difference from the control group in body weight increase or in the intake of food and water.

The mean intensities required to induce the nociceptive responses in control animals were: 0.41 ± 0.02 mA for tail withdrawal, 0.92 ± 0.07 mA for vocalization, and 1.42 ± 0.11 mA for vocalization after discharge. No significant differences were found between data recorded on the different days in any of the responses, $F(2, 30) = 1.287$, $p < 0.29$; $F(2, 30) = 0.832$, $p < 0.44$; and $F(2,30) = 1.74$, $p < 0.19$; respectively.

No differences were found between the control and vehicle groups in the analgesic thresholds or in the behavior parameters. To simplify the exposition of the results the statistical data will be referred to the Tween-treated group.

The treatment with nifedipine or nimodipine induced a significant modifications in the ratio I_3/I_B and I_{11}/I_B ratios for tail withdrawal, $F(7, 181) = 4.218$, $p < 0.0002$. Compared with the corresponding ratios registered in Tween-treated rats, post hoc analysis indicated that the difference reached statistical significant value for all the groups treated with calcium antagonists except for those treated with nifedipine 400 μg/kg/day (Fig. 1A).

No major differences were found for vocalization, $F(7, 181) = 2.092$, $p < 0.05$. Post hoc analysis showed significant

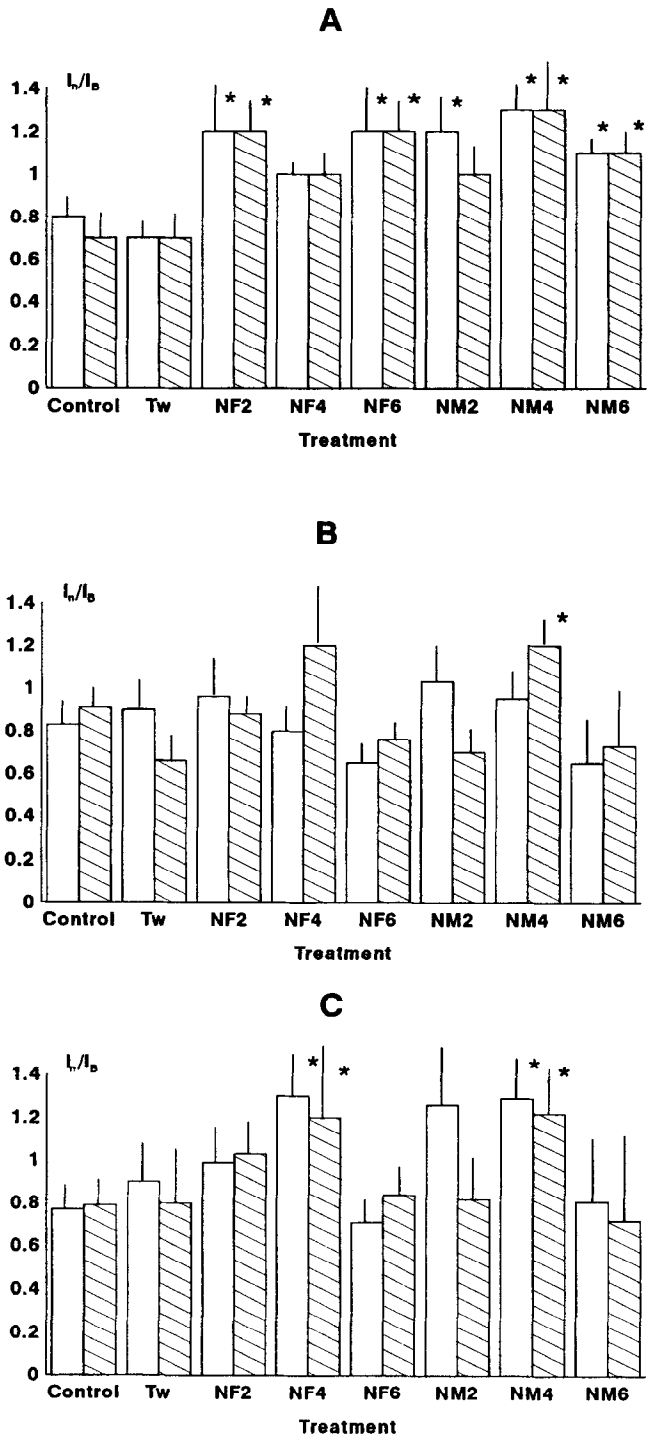

FIG. 1. Analgesic effect of the calcium antagonists evaluated as A—tail withdrawal, B—vocalization, and C—vocalization after discharge. The bars represent the mean of the ratio of the nociception evaluated on days 3 (white bars) or 11 (striped bars), and the nociception evaluated on day −3 in each animal. Rats were treated with: saline—control group (n = 11), Tween (TW) (n = 10), nifedipine (NF) 200 (n = 14), 400 (n = 12), or 600 (n = 12) μg/kg/day, or nimodipine (NM) 200 (n = 10), 400 (n = 12), or 600 (n = 12) μg/kg/day. Vertical lines represent SEMs. *Indicates a significant ($p < 0.05$) difference from Tween-treated group.

TABLE 1

EFFECT OF CALCIUM ANTAGONIST TREATMENT ON HEAD DIPPING AND REARS

Treatment (μg/kg/day)	Head Dipping (sec)	Head Dipping (n)	Rears (n)
Control	27.9 ± 2.7	11.1 ± 1.0	24.7 ± 2.5
Tween	25.7 ± 4.5	9.5 ± 1.0	18.1 ± 2.1
NF (200)	30.1 ± 2.4	11.6 ± 0.9	30.0 ± 2.9
NF (400)	22.5 ± 1.1	11.9 ± 0.9	33.0 ± 2.9*
NF (600)	22.6 ± 3.1	9.0 ± 1.0	24.7 ± 2.9
NM (200)	16.4 ± 4.2	7.4 ± 1.9	20.7 ± 2.5
NM (400)	20.0 ± 2.9	11.1 ± 1.3	25.4 ± 2.5
NM (600)	18.1 ± 2.7	9.3 ± 0.7	20.0 ± 1.7

Values show the mean ± SEM of the time (sec) and number (n) of head dipping and number (n) of rears in control, and in Tween-nifedipine (NF)-, or nimodipine (NM)-treated rats. * Indicates a significant ($*p < 0.05$) difference from Tween-treated group.

differences only between nimodipine 400 μg/kg/day-treated animals and the Tween-treated group (Fig. 1B).

Statistically significant differences were found for vocalization after discharge, $F(7, 181) = 2.37$, $p < 0.02$. Post hoc analysis showed that the difference vs. Tween-treated group was significant only for the groups treated with nimodipine or nifedipine 400 μg/kg/day (Fig. 1C).

When the factor evaluated was the day, no significant differences were found: for tail withdrawal $F(1, 181) = 0.104$ $p < 0.75$, for vocalization $F(1, 181) = 0.310$ $p < 0.59$, and for vocalization after discharge $F(1, 181) = 0.200$ $p < 0.66$.

Treatments with nifedipine or nimodipine decreased slightly the time of head-dipping, $F(7, 97) = 2.389$, $p < 0.026$. The difference from the Tween-treated group was no significant (Table 1). No major variations, $F(7, 97) = 1.922$, $p < 0.74$, were found in the mean number of head dipping (Table 1).

The number of rears was scarcely modified, $F(7, 97) = 3.11$, $p < 0.005$. Post hoc analysis demonstrated that the difference vs. Tween-treated rats reached a statistically significant value for the group treated with the lowest dose of nifedipine.

The number of peripheral crossings did not show statistically significant modifications, $F(7, 95) = 1.938$, $p < 0.07$ (Fig. 2A). The number of central crossings was modified, $F(7, 95) = 6.696$, $p < 0.00001$, a dose-dependent decrease was found, and the difference vs. the Tween-treated group was statistically significant for all the calcium antagonist-treated groups except for nifedipine 200 μg/kg/day (Fig. 2B).

DISCUSSION

The acute analgesic effect of calcium antagonists has been widely investigated in recent years. Acute administration of calcium antagonists (verapamil, diltiazem, nimodipine or nifedipine) potentiate or prolong the analgesia induced by opiates and opioids in control and in morphine-tolerant animals (3,6,7,14,24).

Direct antinociceptive effects of calcium antagonists are controversial and previous data demonstrate that the analgesic effect is depending on the used test. So, when chemical nociceptive stimuli were applied, the administration of calcium antagonists induces analgesia as well as using the writhing test (32,38) or the formalin test (22,31). On the contrary, when thermal nociceptive tests were used results were not so consistent: calcium antagonists do not induce a high level analgesia in the hot plate test (31), and there are data supporting (49) and refusing (14,34) the analgesic effect with the tail flick test.

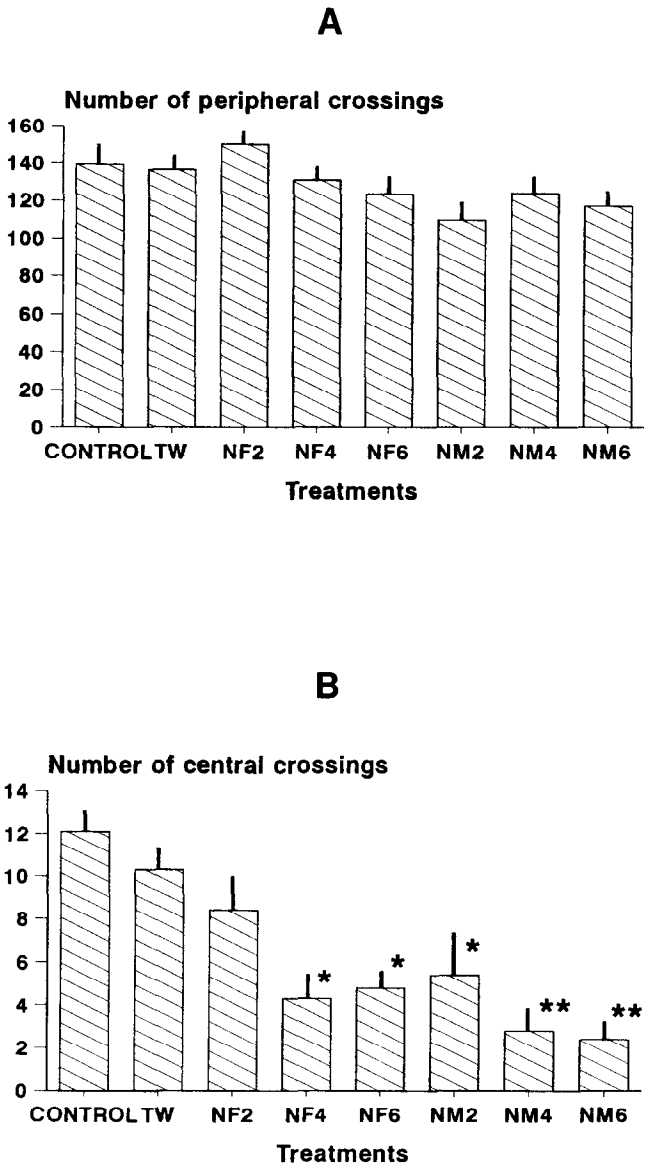

FIG. 2. The bars represent the mean of the number of peripheral (A) and of central (B) crossings in groups of at least 10 rats treated with: saline—control group, Tween (TW), nifedipine (NF), or nimodipine (NM) 200, 400, or 600 μg/kg/day. Vertical lines represent SEMs. *Indicates a significant ($*p < 0.05$, $**p < 0.01$) difference from Tween-treated group.

Our results show that the continuous administration (3 or 11 days) of nifedipine or nimodipine induced analgesia that was mainly evident when tail withdrawal was considered. The involvement of spinal mechanisms in the tail withdrawal has been previously well established (12,30,41) and recently confirmed by Illich et al. (26) in spinalized rats. Present data suggest that the analgesia observed after the administration of these calcium antagonists may be mediated primarily by spinal mechanisms, agreeing with previous results (49). Spinal mechanisms seem to be also involved in the analgesia induced by calcium antagonists blocking N-type channels (5,42).

On the other hand, we found that neither the vocalization nor the vocalization after discharge were modified after the treatment with the calcium antagonists; the lack of evidence of antinociception at supraspinal levels do not permit discarding the involvement of central neural structures in the analgesic effects of the calcium antagonists. The analgesia induced by the intracerebroventricular administration of this drugs in mice using the writhing test (32), together with the potentiation of the opioid analgesia (14,38) and the responsiveness to pain in the hot plate test (2) suggest the involvement of supraspinal structures.

The diverse parameters measured in the holeboard test allow the evaluation of different components of spontaneous behavior, which may be caused by different tendencies or drives (44). Present results indicate that, in general, chronic treatment with either nifedipine or nimodipine does not modify, in a biologically significant manner, the frequency or duration of head dipping, two parameters that are indicative of site directed exploration (1,44). In fact, despite the treated animals showing a significantly lesser tendency to cross the central area of the apparatus, this was not accompanied by a lower exploration of the holes placed just around this area, except for nimodipine at the maximum dose.

The dose-dependent tendency to avoid the central area of the field shown by the animals treated with the calcium antagonists indicates a disruption of the spatial pattern of locomotion. This effect can be interpreted in terms of emotionality or anxiety. The distinction between external and internal ambulation as different parameters, linked with general locomotion or activity and emotionality, respectively, has usually been accepted in studies in open field test (20,29,40,46), and also in studies on holeboard field (1). In both cases, a decreased internal ambulation can be interpreted as an increased emotional level, given the characteristic thigmotaxis of the rat. It is generally accepted that anxiogenic or anxiolytic drugs may modify the pattern of locomotion without significant changes in the total locomotion scores. Anxiety induces thigmotaxis, manifested as the preference for peripheral areas, and this is a robust and reliable defensive behavior that is selectively blocked by anxiolytic agents (45).

Measures of the spontaneous locomotor activity of rats have frequently been used to assess the behavioral effects of drugs in a wide number of physiological and pathologic conditions (35). Acute administration of calcium channel blockers has no effect on the motility of the rats, although they are able to antagonize BAY K 8644-, amphetamine-, or morphine-induced hypermotility (8,21,28). In line with the effects previously described after acute administration, we did not observe significant changes in the number of peripheral crossings after continuous administration. In agreement with the absence of modification in the number of peripheral crossings, our data show no major modifications in the number of rearing. In the holeboard, rearing behavior is considered as another index of general activity (36).

Taken together the absence of modifications in peripheral motility and the increased tendency to avoid the center of the field, displayed by treated rats, imply a disruption of the spatial patterns of locomotion. This condition may agree with the hypothesis of an increase in the emotivity of the rats.

In summary, it is interesting to remark that the chronic administration of nifedipine or nimodipine can induce behavioral changes in rats, suggesting the possibility that calcium channels have neuromodulatory or regulatory actions on the CNS.

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