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Oral administration of nimodipine accelerates functional recovery following peripheral nerve damage in the rat

C.E.E.M. van der Zee[1], T. Schuurman[2], J. Traber[2] and W.H. Gispen[1]

[1]*Division of Molecular Neurobiology, Rudolf Magnus Institute for Pharmacology and Institute of Molecular Biology and Medical Biotechnology, University of Utrecht, Utrecht (The Netherlands) and* [2]*Neurobiology Department, Troponwerke, Cologne (F.R.G.)*

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Oral administration of the Ca^{2+}-entry blocker nimodipine accelerates in a dose-dependent manner the recovery of sensorimotor function following a crush lesion of the rat sciatic nerve. The beneficial effect of nimodipine was apparent in both a foot shock withdrawal test and in a test analyzing the walking pattern of the rat. These data are the first demonstration of nimodipine-induced enhanced recovery following peripheral nerve damage.

A wide variety of studies have documented neuro- and psychopharmacological effects of the Ca^{2+}-entry blockers of the nimodipine-type in animal and man (see ref. 3). As nimodipine increases cerebral blood flow [12, 16], it has been tested for an eventual beneficial effect in brain ischemia. Evidence is accumulating that both pre- and post-treatment with nimodipine of experimentally induced brain ischemia in dogs and monkeys improved post-ischemic cerebral blood flow and neurological function [12, 13]. Neuropathological studies suggested that pretreatment with nimodipine reduced the size of the lesion following occlusion of the middle cerebral artery in rats [12].

The precise mechanism of action of nimodipine by which it brings about the anti-ischemic effects is still largely unknown. It is assumed, however, that protection from cell loss in the afflicted region contributes to the limitation of the functional deficit following ischemia [10]. It may be that nimodipine enhances the supply of oxygen and nutrients to the injured region. On the other hand, as nimodipine passes the blood–brain barrier [17] and binds to specific dihydropyridine receptors in the brain

Correspondence: W.H. Gispen, Division of Molecular Neurobiology, Institute of Molecular Biology and Medical Biotechnology, University of Utrecht, Padualaan 8, 3584 CH Utrecht, The Netherlands.

parenchyma [2, 8], anti-ischemic effects may also originate from the blockade of the slow Ca^{2+} channel active under depolarized conditions [11] possibly reducing an increase in intracellular Ca^{2+} levels provoking cell death [9].

By using a computer-controlled spinal cord impact injury device, Beattie et al. [1] showed that nimodipine treatment reduced the functional deficit and concomitantly increases the extracellular Ca^{2+} level in the injured region. These and other data prompted us to evaluate the potential beneficial effect of nimodipine in the functional recovery following peripheral nerve damage. In the present paper we report that oral administration of nimodipine facilitates recovery of sensorimotor function following a crush lesion of the sciatic nerve in rats.

Female rats of an inbred Wistar strain (CpB, TNO, Zeist, The Netherlands; 140-150 g body wt.) were used. A crush lesion was placed in the right sciatic nerve in the upper leg according to De Koning et al. [4].

From return to their home cages following the surgery onwards, rats received either control food pellets or pellets containing nimodipine. Pellets were prepared by Sniff (Soest, F.R.G.) and contained either no nimodipine or 225 or 860 ppm nimodipine. The dosages were chosen in accordance with the study of Schuurman et al. [15] reporting on the influence of the oral administration of nimodipine on motor performance in senescent rats. The experimentor did not know which rat received what sort of food pellet. Upon completion of the functional tests the treatment code was opened.

Sensitivity to local noxious stimulation of the foot sole was assessed using a slightly modified version of the test procedure described recently [4]. In short, in this test a small electric current is applied to the foot sole through two stimulation poles. A normal rat invariably retracts its paw instantaneously as soon as the skin of the foot sole closes the electric circuit. In the original description of the test method only one current strength was used for testing. In the present study a range of current strengths was applied – 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 mA – and the lowest current strength at which a rat retracts its paw from the stimulus was determined at a certain day. A rat is considered fully recovered (100% recovery), when it retracts its paw on a stimulus of 0.1 mA, whereas no retraction on a stimulation strength of 0.6 mA indicates complete absence of sensitivity to a noxious stimulus (0% recovery). Withdrawal upon one of the intermediate current strengths is scored as incomplete recovery and expressed as percentage of total recovery. By this modification of the originally described test, the recovery of sensory function can now be graded [5].

An index of sciatic nerve function was calculated from the walking pattern of rats as described [6] In short, after dipping the hind feet in photographic developer (Eukobrom, Tetenal, F.R.G.) rats walk over photographic paper (Ilford, 2.24 M, semimatt), which lies on the bottom of an illuminated, 50 cm confined corridor towards a dark goal box. The corridor has a slight inclination of about 10° with the horizontal plane. Foot prints show up immediately after the rat has passed the corridor. From these walking patterns different parameters are measured. (i) Distance between opposite feet: this value is measured orthogonally from the tip of the normal foot to the tip of the following contralateral toes. The value between the tip of the foot print

at the crushed side to the tip of the following foot on the normal side. (ii) Print length: the length of the longest foot print on both the normal and the crushed side was measured. (iii) Toe spreading: the linear distance between the first and fifth toe and between second and fourth toe was measured on the normal and crushed side.

Entering the values of these variables from each side into an empirically derived formula, yields an index of motor function of the paw bearing the crush lesion in relation to the function of the contralateral uncrushed control paw (functional sciatic index, FSI). The value of the FSI is set at -100% at day one following surgery and in time the motor function returns to normal (FSI $\pm$ 10%). For further details see refs. 5 and 6.

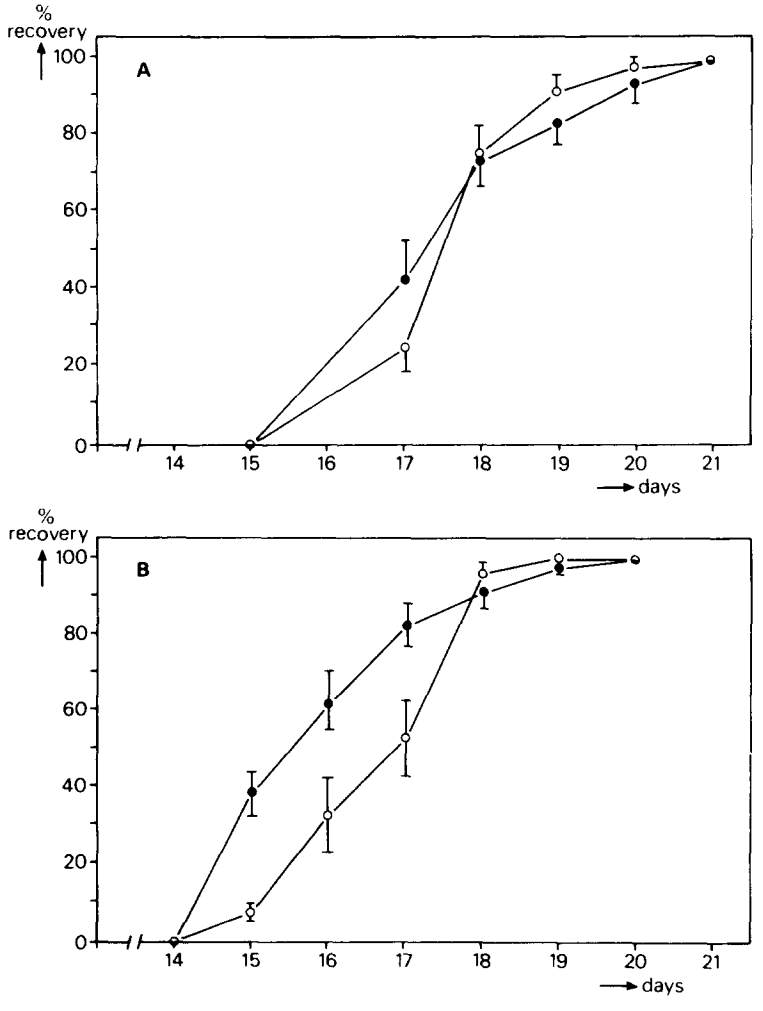

Fig. 1. The effect of oral administration of nimodipine (A, dose 225 ppm; B, dose 860 ppm) on foot reflex withdrawal in response to a local foot shock, following a sciatic nerve crush ($n = 10$ for each group). Open circles, control; closed circles, nimodipine (mean % recovery $\pm$ S.E.M.).

The data were statistically analyzed by an analysis of variance for repeated measures followed by a supplemental t-test.

In the first experiment 20 rats received a crush lesion in their right sciatic nerve. Nimodipine was administered via food pellets (225 ppm) to 10 rats, whereas the other 10 received control food pellets, not containing nimodipine. Return of sensory function was measured from post-operation day 11 onwards every other day, and daily from day 17 through 21. As can be seen in Fig. 1A, the first signs of recovery of function were observed between day 15 and 17 and functional recovery was complete as assessed by the foot withdrawal reflex by day 20. However, no effect of oral administration of nimodipine (225 ppm) was detected. Analysis of motor function of these rats was performed by calculating the FSI from their walking pattern at post-operation days 6, 10, 14, 16, 18 and 20. As shown in Fig. 2A, the FSI of the experimental paw in the control group reached normal levels from day 18 onwards. Furthermore, at all days tested, the FSI of nimodipine-treated rats was smaller than that observed in the untreated rats. Analysis of variance revealed significant enhancement of recovery of function in nimodipine-treated group ($F_s = 4.60$; df = 1, 18; 2 $P < 0.05$).

In the second experiment a larger dose of nimodipine was tested (860 ppm). Again 10 rats received control food pellets and 10 rats nimodipine-containing pellets. The sensory function was tested daily from post-operation day 13 through 20. The walking pattern was analyzed at post-operation days 2, 6, 12, 14, 16, 18 and 20. As can be seen in Fig. 1B in untreated rats first signs of recovery of sensory function were observed at day 15. Nearly total recovery was observed from day 18 onwards. As

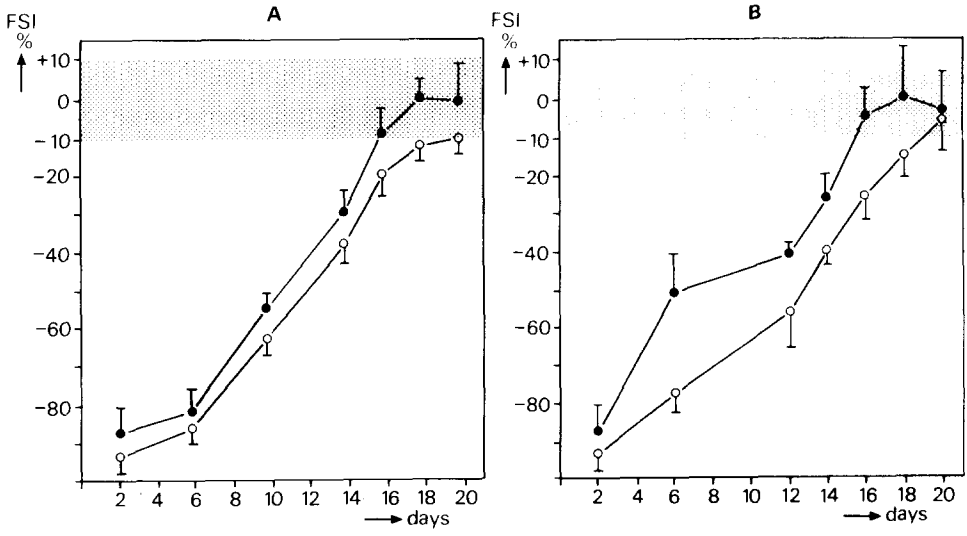

Fig. 2. The effect of oral administration of nimodipine (A, dose 225 ppm; B, dose 860 ppm) on the functional sciatic index (FSI) following a sciatic nerve crush. FSI was calculated from the walking pattern of the rats ($n = 10$ for each group). Open circles, control; closed circles, nimodipine (mean % FSI ± S.E.M.). Shaded area represents FSI values of unoperated rats.

illustrated in Fig. 1B, at this higher dose level nimodipine significantly enhances the return of sensory function following sciatic nerve damage (days 15–17; $F_s = 14.27$; df = 1, 18; 2 $P < 0.001$). Also in the motor function test the effect of a higher dose of nimodipine was more pronounced (Fig. 2B). Non drug-treated rats showed a normal FSI from post-operation day 18 onwards, whereas at all days tested the nimodipine group showed smaller FSI values (days 6–18, $F_s = 16.02$; df = 1, 18; 2 $P < 0.001$).

Previously we have shown the reliability of the experimental procedures used. The distance between the position of the crush and the sciatic notch was 26.5 ± 0.3 mm ($n = 3$, mean $\pm$ S.E.M.). Furthermore, it was demonstrated that shortly following the crush in the distal segment of the nerve all fibers were degenerating [18]. In addition, the tests for return of sensorimotor function were proven to be sufficiently accurate and sensitive to allow screening of efficacy of treatment with potentially neurotrophic compounds (melanocortins, $ACTH_{4-10}$, the $ACTH_{4-9}$ analog Org. 2766, α-MSH, gangliosides) [4, 7, 18]. We are aware of the fact that the sciatic nerve is a mixed sensorimotor nerve and that in both functional tests used sensory as well as motor modalities of the paw contribute to the response measured. In keeping with the literature, the foot shock withdrawal reflex is taken to measure the sensory modality whereas the walking pattern is taken to describe the motor function of the paw. The advantage of the latter test is that it describes the unforced use of the paw in walking and therefore provides evidence on the quality of the functional recovery [5].

For reasons given in the introduction, these experimental procedures were used to test the efficacy of the Ca^{2+}-entry blocker nimodipine to influence the functional recovery following peripheral nerve damage. Although nimodipine is a non-polar, poorly water-soluble compound, previously it was demonstrated that oral administration results in complete and rapid absorption in the gastrointestinal tract [14]. The present study shows enhancement of recovery of function of peripheral nerve damage following oral administration via ad libitum intake of food pellets containing nimodipine.

The data presented in Fig. 2 seem to suggest that nimodipine, like Org. 2766 [4] does not facilitate the rate but rather affects the onset of the recovery process. As is the case with the beneficial actions of nimodipine seen following brain and spinal cord damage [1, 12, 13], the mechanism by which nimodipine enhances functional recovery is still unknown. It may be that vascular effects at the site of the lesion are part of the neurotrophic efficacy of this drug. Furthermore, the crush procedure as used in this study was shown to affect all neurites at the crush site, and thus, it seems unlikely that reduction of secondary cell loss is responsible for the observed effect. Currently, the potential neurotrophic properties of nimodipine on peripheral nerve repair mechanisms are studied at the histological and neurophysiological level.

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