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CNS and PNS effects of nimodipine

P. R. Bär[1,3], J. Traber[2], T. Schuurman[2], and W. H. Gispen[3]

[1]Research Laboratory, Department of Neurology, University of Utrecht, The Netherlands
[2]Institute for Neurobiology, Troponwerke, Köln, Federal Republic of Germany
[3]Department of Pharmacology, Rudolf Magnus Institute, University of Utrecht, The Netherlands

Summary. Calcium ions play a decisive role in a large number of processes dealing with development, regeneration after damage, and aging of elements of the nervous system.

Nimodipine, a member of the 1.4-dihydropyridine chess of calcium-channel blockers, has proved to influence the calcium metabolism of neurons.

This review focusses on the results of recent studies on the role of nimodipine in both the central and peripheral nervous system, during or after events that threaten the integrity or function of the nervous system.

Introduction

Neural plasticity may be defined as the capacity of the nervous system to adapt continuously to changes in its internal or external environment. It can be studied at the molecular, morphological, neurophysiological and behavioral level. Neural plasticity is an essential characteristic of neural function, and is observed in both peripheral and central nervous system. For instance, the neuromuscular junction of mammalian skeletal muscle fibers is modified throughout life in a process called synaptic remodelling, as described for both animal (Barker and Ip, 1966) and man (Wokke et al., 1990). Other studies showed that in the normal adult frog, the nerve endings sprout continuously (Werning et al., 1980). Recent studies of the plasticity of cortical sensory maps indicate that map organization can also be modified during adult life (Wall, 1988; Wall and Kaas, 1985). From these observations it can be stated that the nervous system is dynamic rather than static, and that adaptive changes occur at all levels of complexity.

Neural plasticity is of great importance in relation to a number of serious human health problems such as recovery after injury to the peripheral nerve, spinal cord or brain, developmental disorders, and dementia (Gelijns et al., 1987). The mental decline associated with brain aging is commonly considered to result, at least in part, from a reduced neural plasticity. To better understand, and possibly treat these conditions, further insight into the

mechanisms underlying neural plasticity is of vital importance. It has become evident that neural Ca^{2+} homeostasis is a key factor in the control of plasticity. Evidence is accumulating to suggest that intracellular Ca^{2+} is a major factor in the regulation of growth cone motility. Different growth cone behaviours such as protrusion, retraction and elongation seem to have different Ca^{2+} dependencies (Kater et al., 1988). Furthermore, the synaptic plasticity that can be observed during the establishment of long-term potentiation in rat hippocampal synapses involves Ca^{2+} entry (Malenka et al., 1989), activation of the so-called NMDA (N-methyl-D-aspartate) receptors to which Ca^{2+} channels are linked (Collingridge and Bliss, 1987), activation of Ca^{2+}-sensitive processes including phosphatidylinositol 4,5-biphosphate breakdown (Bär et al., 1984), protein kinase C-mediated phosphorylation of specific substrates, such as the protein B-50/F1 (Akers et al., 1986; De Graan et al., 1986) and, finally, the activation of certain Ca^{2+}-activated neutral proteases (CANP) of which the calpains are described recently (Melloni and Pontremoli, 1989). In the aged brain, in which neural plasticity is diminished in such a way that it hampers the individual's normal functioning, Ca^{2+} homeostasis is severely disturbed (Gibson et al., 1984; Khatchaturian, 1984; Landfield, 1988b, see below).

Many observations now exist indicating that Ca^{2+} antagonists can modulate numerous aspects of nervous system function. Recent studies have suggested that nimodipine, a Ca^{2+}-entry blocker of the dihydropyridine class, may reduce functional deficits in spinal cord injuries (Beatty et al., 1986; Fehlings et al., 1989), and improve both peripheral and central nervous system function following trauma or in aged laboratory animals (Schuurman et al., 1987; LeVere and Sandin, 1989). These findings may indicate a therapeutic application of nimodipine in both CNS and PNS dysfunction associated with trauma or aging. In this paper we review data with respect to several aspects of neural plasticity, and the role that nimodipine may play in conditions in which normal plasticity is diminished.

The role of Ca^{2+} in neural plasticity

Ca^{2+} ions play an important role in the regulation of many neuronal functions, and it is well proven that they have a key function in the control of neural plasticity. The extracellular Ca^{2+} concentration is about 1 mM, whereas inside the neuron, and indeed inside most other cells, the Ca^{2+} concentration is 10,000 times lower, about 0.1 μM. The large number of tools that are at the cell's disposal to remove, bind and sequester Ca^{2+} (Simons, 1989), are indicative of the utmost importance for a cell to control and fine-tune its free Ca^{2+} concentration. In order for Ca^{2+} to act properly as second messenger, the intracellular Ca^{2+} concentration must be low, so that transient Ca^{2+} peaks, elicited by extracellular signal molecules such as hormones and neurotransmitters, can be picked up. Thereafter, the intracellular Ca^{2+} concentration must be rapidly restored to basal levels, so that a next peak will not be missed. The intracellular Ca^{2+} concentration can rise as a result of release of

Ca^{2+} from intracellular stores, from Ca^{2+} bound to membrane surfaces and cytosolic components, and from organelles in which Ca^{2+} ions are segregated. Several chemical stimuli (e.g. hormones and neurotransmitters) act upon cell surface receptors and stimulate the breakdown of the phospholipid phosphatidylinositol biphosphate, which in turn generates diacylglycerol (DAG) and inositol triphosphate (IP3). Activation of the intracellular receptor for IP3 leads to release of Ca^{2+} into the cytoplasm (Berridge, 1984). The other major cause of increased free cellular Ca^{2+} is the opening of voltage-sensitive Ca^{2+} channels (VSCC) on the plasma membrane through which Ca^{2+} can pass into the cytoplasm. Recent electrophysiological studies have provided evidence that there are at least three types of VSCC, the L-, N- and T-type (Tsien et al., 1988). Thus, the free Ca^{2+} within synapses is modulated by electrical and chemical stimuli, thereby altering synaptic function via a series of Ca^{2+}-sensitive modulating steps.

In addition to its role in the function of pre-existing synapses, Ca^{2+} is important in the remodelling of neuronal connections. Formation of new synapses involves growth and is dependent on a specialized region, known as the growth cone, located at the tip of the growing nerve process. Intracellular Ca^{2+} is of major importance in the regulation of growth cone motility (Kater et al., 1988). Changes in the membrane potential, caused by a variety of stimuli, are integrated at the level of the growth cone membrane. Voltage-gated Ca^{2+} channels transduce the net effect on the membrane potential into an intracellular Ca^{2+} pulse. The free Ca^{2+} level within the growth cone acts as a final integrator of the environmental stimuli by influencing the elements (cytoskeleton and vesicular apparatus) that underlie its motility (Kater et al., 1988). The effects of Ca^{2+} on the cytoskeleton elements may be direct or may require intervening Ca^{2+}-binding proteins, such as Ca^{2+}-dependent kinases and proteases. Intracellular levels of free Ca^{2+} have been shown to be lower in spontaneously inactive growth cones than in those that were active. Apparently, the balance between influx, efflux and Ca^{2+}-buffering systems such as pumps, Ca^{2+}-binding proteins and organelle sequestering systems, play a crucial part in the regulation of growth cone behaviour. Both excessive increases and excessive decreases in cellular Ca^{2+} impair growth cone function and thus the ability of the axon to reach its proper target (Kater et al., 1988).

There are indications that nerve terminals at the end plates of skeletal muscle fibers are being remodelled throughout life (Barker and Ip, 1966; Tuffery, 1971; Werning et al., 1984; Wokke et al., 1990), and these changes seem to be, to some extent, related to the activity of the synapse (Grinnel and Herrera, 1981). It has been reported (Connold et al., 1986; Swanson and Vrbova, 1987) that the activity-induced elimination of nerve-muscle contacts is dependent on the Ca^{2+} concentration and regulated by CANP. By increasing the Ca^{2+} concentration, nerve-muscle contacts are disrupted and this effect of Ca^{2+} was found to be prevented by concurrent inhibition of the CANP. Conversely, reducing Ca^{2+} or inhibiting CANP activity reduces the rate of the naturally occurring elimination of polyneural innervation. Adult mammalian nerve endings are known to continue extending and retracting small branches (Grinnel and Herrera, 1981) and it may be that the retracting

branches are normally removed by CANP activity, which itself is activated by Ca^{2+} entry (Swanson and Vrbova, 1987).

Another area in which plasticity of neural tissue plays an important role is that of neural grafting. This is in fact a "classical" field of research, going back to the 19th century (Thompson, 1890; reviews: Björklund and Stenevi, 1985; Gash, 1984). It became clear that the age of the donor is an important factor in producing successful transplants: when embryonic tissue is grafted, it not only survives, but may also form functional connections with the host brain (Das and Altman, 1971; Lund and Hauschka, 1976; Stenevi et al., 1976). Probably of equal importance are characteristics of the site into which the tissue is placed; of these, the vascularization of the immediate environment of the graft may be decisive for a viable transplant. This was already recognized in 1917 by Dunn who saw "retention in place" (of the material transferred) and "furnishing to it of an adequate blood supply" as the main factors in successful cerebral grafting (Dunn, 1917). This early observation is confirmed by most authors that attempt grafting of intact pieces of tissue. The property of the calcium antagonist nimodipine to dilate (cerebral) blood vessels, thus increasing blood flow to treated areas, has led several experimentators to treat the receiving animal with nimodipine. Finger and Dunnett (1989) used animals with a lesion in the nigrostriatal bundle, often employed as a model for Parkinson's disease. They administered a suspension of embryonic dopamine-rich cells (from the ventral mesenphelic area), a technique in which the blood supply to the new tissue is less critical, as the dissociated donor cells "spread out" more, thus being more accessible to the existing blood supply. It appeared that when grafting was performed under suboptimal conditions, i.e. when the cell suspension was made from tissue from older donor embryo's, and with a long delay between preparation of the cell suspension and transplantation, nimodipine had the most beneficial effect: a better vascularization and a significant "growth" of the graft. Grafts performed under optimal conditions could not significantly be improved. This observation has important implications for clinical situations, where often the conditions cannot be rendered optimal as easy as in the laboratory (Finger and Dunnett, 1989).

Neuroprotection at the cellular level

As explained above, the role of Ca^{2+} is pivotal in neuronal differentiation and neuronal death. Tissue culture of primary neurons provides a unique opportunity to observe direct effects of drugs *in vitro*. It is especially convenient when essential ions or molecules are suspected of having trophic or toxic actions, and when the concentrations must be carefully controlled. As stated above, high intracellular Ca^{2+} causes a strong inhibition of protein phosphorylation, the uncoupling of ATP-production from mitochondrial electron transport and an activation of Ca^{2+}-dependent proteases and phosphorylases. These events will ultimately result in the death of the cell (Young, 1987). Thus, disruption of the normal homeostasis of intracellular

Ca^{2+} has been suggested to be the final common pathway of the death of a variety of cell types, including neurons (Schanne et al., 1979; Starke et al., 1986). In neuronal cell cultures, Ca^{2+} plays a crucial role in adhesion of the neurons to their substratum and the formation of neurites. Cerebral cells (derived from fetal mice) were grown for 48 h, either in Ca^{2+}- and serum-free medium, or in Ca^{2+}-free medium with 10% fetal calf serum; they showed a decrease in process formation and membrane ruffling when compared to cells grown in complete media (Young, 1987). After 24 h in culture, the cells grown in Ca^{2+}-free media, with or without serum, showed signs of degeneration. Experiments with molluscan neurons in culture indicated that optimal levels of intracellular Ca^{2+} are a necessity for neurite extension and growth cone movement. Surprisingly, these two processes appeared to have differential sensitivities for Ca^{2+}. A low extracellular Ca^{2+} concentration (0.2 mM) increased neurite extension but reduced the appearance of growth cone filopodiae (Mattson and Kater, 1987). In immature fetal serotonergic neurons, Ca^{2+} spikes proceeded Na^{2+} spikes by several days (Segal and Azmitia, 1986). Thus, manipulation of Ca^{2+} during early gestation can enhance both neuronal survival and neurite growth.

Azmitia (1989) used excitatory amino acids, such as glutamate, to trigger the NMDA receptor in cultures of fetal serotonergic neurons; this cell type is known to have glutamatergic input in the intact brain (Kalen et al., 1985). When the intracellular Ca^{2+} concentration is raised in this way, the development of these neurons in culture is inhibited. Nimodipine proved to be capable of protecting the cells against glutamate neurotoxicity, as described earlier for cortical cells with specific NMDA-receptor antagonists (Choi et al., 1987). In another experiment with the same cells, 3,4-dimethylenedioxymethamphetamine (MDMA, a psychotropic drug) was used as a neurotoxic substance. MDMA most likely produces its neurotoxic effect via a Ca^{2+}-related mechanism, namely by blocking the uptake-linked Ca^{2+} efflux and stimulating the release of intracellular Ca^{2+} stores. Again, nimodipine proved to be neuroprotective against the effects of MDMA. The conclusion of the author is that closing the L-channel allows the neuron to deal more effectively with imbalances in the cytoplasmic levels of free Ca^{2+}, regardless of whether the Ca^{2+} increase is caused by an increased influx, a decreased efflux, or a release of intracellular stores of Ca^{2+} (Azmitia, 1989).

In another series of experiments by Fahey et al. (1989) PC12 cells were used, preloaded with rhodamine-123. This dye is concentrated in mitochondria and released when cells are treated with chemicals known to disrupt mitochondrial function (Lachowiez et al., 1989). Exposition of PC12 cells to mercury or low oxygen conditions resulted in a dose-dependent release of rhodamine-123; verapamil and nimodipine, both Ca^{2+} channel blockers, were effective in protecting the cells against the cytotoxic treatment. However, whereas verapamil was neuroprotective in the micromolar range, nimodipine elicited its optimum effect in the nanomolar range. Both drugs showed a U-shaped dose-response curve: at higher concentrations they were apparently less cytoprotective, and in the hypoxia experiments, even more damage was caused by the blockers than by hypoxia alone in controls. One possible ex-

planation lies in the properties of the voltage-sensitive channels: exposure to the toxic substances might change the response of these channels to different doses of the blockers. This experimental approach, now tested for the non-neuronal, but neuron-like PC12 cell, may be useful in approaching the matter of protection in cultured neurons from different regions of the central nervous system (Fahey et al., 1989).

Robson and Burgoyne (1989) cultured dorsal root ganglion cells from neonatal rats under highly favourable conditions: in the presence of conditioned medium, nerve growth factor and 10% serum, on polylysine- and laminin-coated substrata. Addition of high K^+, veratridine or bradykinin to the culture medium inhibited the outgrowth of the neurons by about 60%, measured in a qualitative yes-or-no fashion: the appearance of small neurites was counted with respect to the total number of viable cells after 12 h in culture. The drugs and K^+ cause depolarization, concurrent with ion shifts over the membrane, resulting in a high intracellular Ca^{2+} level. When the cells were treated with nifedipine, another dihydropyridine compound, the inhibition was reversed and outgrowth returned almost to normal levels. Our own experiments with dissociated spinal cord cells do not show a similar effect of nimodipine. The difference may be explained by the fact that we used different cells, but also by the fact that we cultured under basal rather than highly favourable conditions: the presence of laminin and of NGF may be crucial for the (sensory) neurons of the dorsal root ganglion. It may be that the K^+ challenge in the spinal cord cell culture, which resulted in a large reduction of outgrowth, is too strong to be overcome by nimodipine treatment alone. The L-channel has been proven to be present in high amounts in rabbit dorsal root ganglion neurons (McCarthy, 1989), but not yet with the same technique in the spinal cord. Preliminary results (Bär et al., in preparation) with intact slices of the fetal rat spinal cord show a disturbed neuritic outgrowth after treatment with K^+, as classified with a method described by Van der Neut et al. (1988). When these slices are treated with nimodipine (10^{-6} to 10^{-7} M), the outgrowth is restored to control levels. Further studies are in progress to characterize this effect.

Nimodipine and postlesion plasticity in the CNS and PNS

The neuron is an extremely specialized differentiated cell and has proved to be the most vulnerable cell in the mammalian central and peripheral nervous system. In general, it is assumed that damage to cell bodies of neurons results in irreversible degeneration and cell death. However, many results point to the possibility for neuronal cells to regenerate, be only under special conditions. If damage is restricted to the neuronal processes (dendrites and axons), regeneration with resulting reinnervation of the target is possible. For reasons still not completely understood, the neurons in the PNS regenerate successful whereas neurons in the CNS display a very poor regenerative response.

The CNS

During the last few years, evidence has been accumulating that certain Ca^{2+} antagonists are useful in the treatment of several CNS disorders. The neuronal effect of nimodipine appears to be mediated through slowly inactivating Ca^{2+} channels (L-type) in a voltage-dependent manner; the blockade of these channels leads to reduction of Ca^{2+} entry into neurons (Scriabine et al., 1989). LeVere and Sandin (1989) investigated the effect in rats of repeated administration of nimodipine on the recovery of preoperatively learned behaviour, following a brain lesion known to disrupt that behavior. Nimodipine significantly reduced the number of errors made by rats. The facilitatory effect of nimodipine on recovery has been reported by these authors to be probably due to an effect on the memory process. Isaacson has observed a nimodipine-induced enhancement of recovery, following a brain lesion in rats (personal communication). These observations suggest that nimodipine might be of use in the treatment of brain injuries, in addition to its protective effect in ischaemia.

LeVere's group worked on the hypothesis that if nimodipine can mitigate trauma-induced retrograde amnesia, and if it did so by effecting memory retrieval, then it might also be effective in reinstating memories that are spared, but less accessible following neocortical injury (LeVere and Sandin, 1989). Whether nimodipine acts directly on spared memories or whether it acts more indirectly, was addressed next. Normal rats, and rats with visual neocortical injuries were trained in the brightness discrimination task where nimodipine was found effective in a previous study. Nimodipine had no reliable influence on the original acquisition of this simple visual behavior and the authors concluded that the drug facilitates the recovery of learned behaviors by promoting the effectiveness of those memories that are spared by the injury. The results may indicate that nimodipine can influence the consequences of brain injury at some time after the actual injury (two weeks in these experiments) by influencing the animal's ability to access spared memories. Their data parallel observations that nimodipine can mitigate certain learning disabilities associated with aging (Deyo et al., 1989) but not the learning abilities of younger animals. It is suggested that the drug is effective in aged animals, because it acts on memory utilization rather than on acquisition (see also below).

A recent report from Fehlings et al. (1989) describes the effect of nimodipine treatment, with and without dextran, on an experimental acute spinal cord lesion. The rationale behind this experiment is that there is evidence that two separate mechanisms are involved in this type of injury: the initial mechanical damage, and secondary changes due to vascular or biochemical effects. The authors measured both spinal cord blood flow and motor- and sensory-evoked potentials to evaluate a possible beneficial effect of nimodipine (0.02 and 0.05 mg/kg) and dextran 40, alone or in combination. Dextran 40 plus 0.02 mg/kg nimodipine was the only combination that resulted in a significant increase in blood flow at the site of injury (at Thoracale 1), as well as an improvement of both electrophysiological parameters. Not only do

these results indicate that an increased blood flow in the damaged area may improve the function of the damaged cord, they also give strong support to a role for the hypothetical posttraumatic ischaemia in the pathogenesis of acute spinal cord injury.

The PNS

The effects of nimodipine on plasticity of the peripheral nerve have been studied in rats in a crush model. Return of both sensory and motor function can be monitored easily by applying techniques described by De Koning et al. (1986). The speed of recovery can be assessed by using foot flick withdrawal, and the quality of recovery can be monitored with an analysis of the free walking pattern. Van der Zee et al. (1987) reported that orally administered nimodipine enhanced recovery of both sensory and motor function in this model, reducing the number of days needed for recovery by 2–3 days, and improving the walking pattern. In a separate experiment, a crush lesion was produced unilaterally in the sciatic nerve of four groups of rats. Animals received 5, 10 or 20 mg/kg of nimodipine via intraperitoneal injections every 48 h, starting on the day of the crush. The control group was injected with vehicle (polyethyleneglycol/saline 2:1). The quality of recovery was assessed by analyzing the free walking pattern. In all three nimodipine-treated groups the sciatic functional index (SFI) returned to normal earlier than in the control group. The rats treated with 10 and 20 mg/kg showed a better overall recovery than the controls. Improvement of walking pattern was significantly faster only in the high-dose groups (Fig. 1).

Mechanical injury is but one of a broad spectrum of conditions that present a potentially life-threatening hazard to the vulnerable neuron. A substantial number of compounds can induce neurotoxicity that is then manifested by a peripheral polyneuropathy. Neurotoxic chemicals include not only environmental and occupational hazards such as heavy metals and organic solvents, but also drugs such as vincristine and cisplatin (Spencer and Schaumburg, 1984). The latter is effectively used in the treatment of ovarian and testicular cancer. At present, cisplatin-induced neuropathy is a major and dose-limiting side-effect. After cessation of treatment the neuropathy is only partially reversible (Roelofs et al., 1984). In order to study the effects of cisplatin on the PNS, and to investigate compounds that might be of benefit in the prevention and treatment of this neuropathy, an animal model of drug-induced polyneuropathy has been established (De Koning et al., 1987). Rats are given cisplatin in a dose of 1 mg/kg, twice a week by intraperitoneal injection for a period of 12 weeks or more. During this treatment the animals lose weight, but the morbidity is low. The motor (MNCV) and sensory (SNCV) nerve conduction velocities can be measured by stimulating the sciatic nerve consecutively at two points with percutaneous needle electrodes and recording the muscle action potential from the plantar muscles of the foot in an anaesthetized rat. This can be repeated a number of times, so that every animal serves as its own control. After a cumulative cisplatin dose of 13 mg/kg the

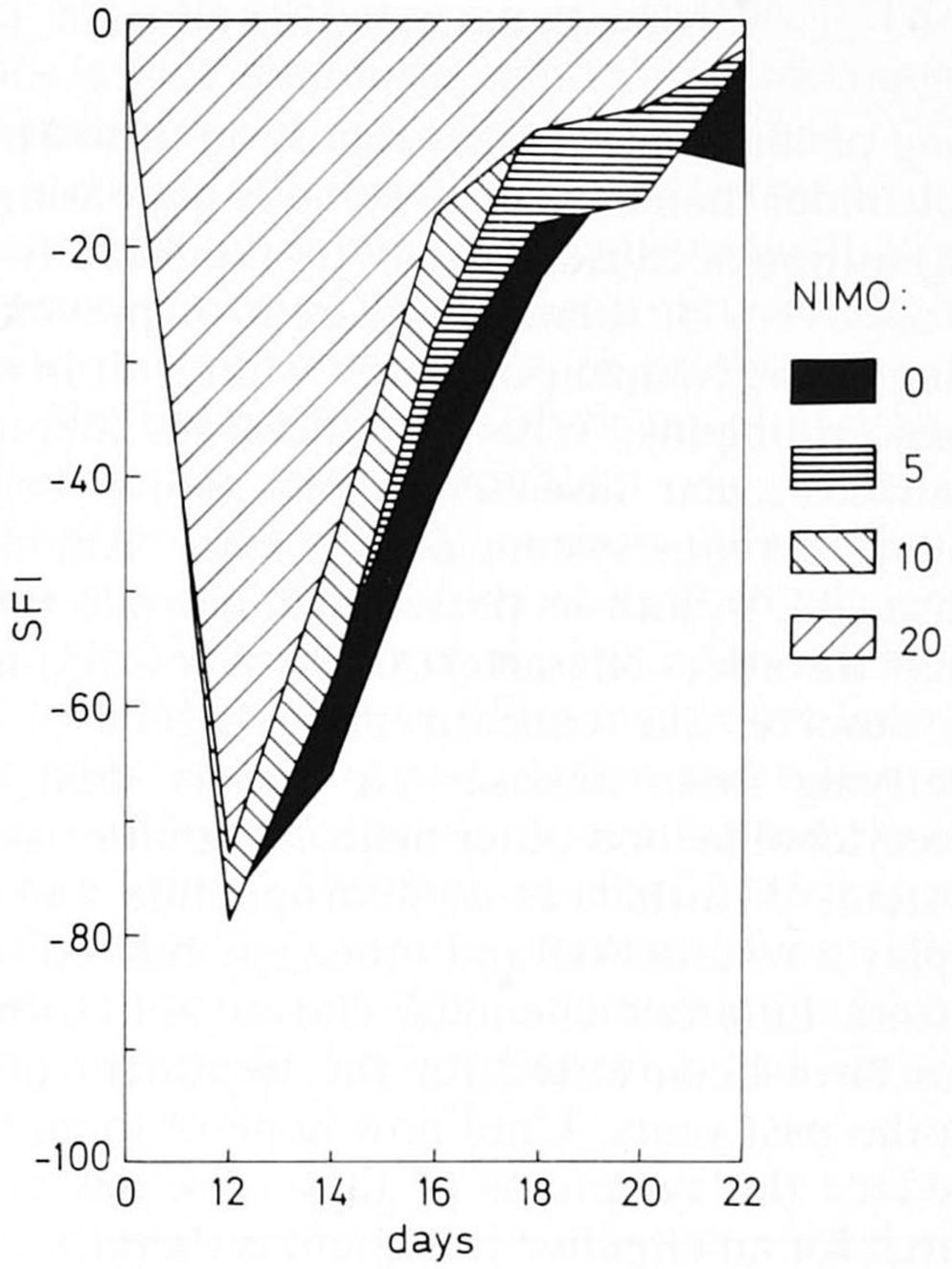

Fig. 1 Recovery of the SFI (sciatic functional index) after a crush of the N. ischiadicus with 3 doses of nimodipine (5-10-20 mg/kg), compared to untreated controls (CO)

SNCV is significantly lower than that in control untreated rats. The SNCV further decreases in a dose-dependent manner. When the administration of cisplatin is stopped, a slow but complete recovery is seen.

This model was used to study the possible beneficial effect of nimodipine on cisplatin-induced neurotoxicity. One group of rats received nimodipine orally, dispersed into food pellets in a concentration of 1000 ppm. Another group was fed control food pellets. After a cumulative dose of 13 mg cisplatin, the SNCV of the rats given control food was 20% lower than that of nimodipine-fed rats. This difference became more pronounced with an increasing cumulative dose of cisplatin. No difference was seen between nimodipine-treated rats and normal age controls. The experiment strongly suggests that nimodipine can prevent cisplatin-induced neurotoxicity in rats. This observation may have consequences for treatment of different types of cancer with cytostatics such as cisplatin, vincristin etc. If the side effects of these drugs can be decreased, treatment of the tumor, which is often limited by the side effects, can be more effective. Unfortunately, the mechanism of toxicity of cisplatin, and indeed of most other neurotoxins, is not known. It has been proposed in 1979 (Schanne et al., 1979; see also Starke et al., 1986, and Komulainen and Bondy, 1988) that toxic substances kill cells in at least two steps, the last being an elevation of free intracellular Ca^{2+} as "final common pathway" leading to the toxic death of cells.

Nimodipine and aging

Since the beginning of this century the proportion of the human population consisting of people older than 65 years is steadily increasing. This shift in the demographic composition is caused mainly by the steadily decreasing birth-rates and by the discovery of therapies to treat or prevent life-threatening diseases. This aging of the human populaton will result in medical as well as social and economic problems. Whereas efficacious therapies for bacterial infections and cardiovascular diseases of old people exist, therapies for diseases of the aging nervous system do not exist. Among these diseases, Alzheimer's disease (AD) (both its presenile and senile form), multi-infarct dementia and other disorders characterized by a severe impairment of cognitive functions, deserve our concern most urgently. The pathological mechanisms underlying these diseases are largely unknown. Apart from disturbances of acetylcholine and other neurotransmitter systems such as the serotoninergic system, disturbances of neuropeptide and Ca^{2+} ion homeostasis probably play a role in AD and other age-related disorders.

Many drugs from different chemical classes and with different therapeutical principles have been tested for the treatment of AD and related dementias during the past years. Until now none of them have been proven to significantly reduce the symptoms of these diseases or to prevent their progress. The search for an effective treatment is therefore more urgent than ever. AD seems to be a disease restricted to humans: although the brains of some animal species (monkeys, dogs) do develop plaques and tangles in (very) old age which resemble these markers in the brains of Alzheimer patients, other characteristics of the disease, such as the degeneration of cholinergic cells in the basal ganglia, seem to be confined to humans. Although this somewhat limits the possibility of extrapolating results from animal studies, the behavior of old rats is still often used as a model for the cognitive deficits seen in AD and other dementing disorders.

Schuurman and Traber (1989) compared behavioral aspects of old rats with those of younger animals in several learning- and memory paradigms as has been done before in other species (Bartus et al., 1978; Bartus et al., 1982). Furthermore, comparisons were made in tests to assess sensorimotor functioning, spontaneous behavior in a novel environment and intra-species social behavior, because the behavioral symptoms of AD and senile dementia are not confined to learning and memory deficits. Other affected behaviors or behavioral systems are social behavior, sleep and selfcare. Old rats (24 months) fed nimodipine-containing food for 4 weeks were more active than matched controls. This effect was not the result of a direct, acute effect on psychomotor behavior because single doses of nimodipine do not stimulate spontaneous behavior of young (Hoffmeister et al., 1982) or old rats (Schuurman, unpublished). Long-term nimodipine-food (250 ppm) treatment had also an effect on sensorimotor functions and locomotion in old rats: old rats kept their balance on a small horizontal rod significantly longer than untreated controls. In other sensorimotor tests, at a higher concentration of nimodipine (860 ppm), treated rats performed better than age-

matched placebo controls (Schuurman et al., 1987). The conclusion from these experiments is that long-term nimodipine treatment counteracts the age-dependent impairment of sensorimotor functions.

Spontaneous locomotion is reduced in old rats. In order to study the effects of aging and of nimodipine treatment on qualitative aspects of locomotion, old rats were subjected to a walking pattern test. The test apparatus consisted of an alley giving access to a dark goalbox, into which the rat will walk voluntarily. The bottom of the alley is covered with a sheet of photographic paper. Before the test the hindfeet are dipped into photographic developer, so that prints of the hindfeet show up and can be analyzed. This test has been used successfully to study the effects of damage to the sciatic nerve on walking (De Medicanelli et al., 1982; Gispen et al., 1988; Van der Zee et al., 1987). Several qualitative differences were found between old and young rats: older animals display exorotation and a lack of elevation (dragging), probably caused by a reduction of central coordination of the movements of the hindlegs during locomotion (Fig. 2) (Schuurman et al., 1987). It was demonstrated that the onset of abnormal footprints was significantly delayed in old rats fed with nimodipine-containing food from the age of 24 months on (Schuurman et al., 1987; Schuurman and Traber, 1989; Scriabine

Fig. 2 Footprints of a 28 month-old rat and a similar old rat treated for 16 weeks with nimodipine containing food (860 ppm) [In: Traber J, Gispen WH (eds) Nimodipine and central nervous system function. Schattauer, Stuttgart New York]

et al., 1989). The most effective concentration of nimodipine was 860 ppm, corresponding to a daily intake of approximately 30 mg/kg. Lower concentrations (250 and 500 ppm) were less effective and a higher concentration (1200 ppm) was equally effective in delaying abnormal locomotion.

In another experiment it could be demonstrated that nimodipine treatment (860 ppm) improved the walking pattern of 24 months old rats which already showed abnormalities, so that long-term treatment not only delays the onset of abnormal walking, but also reduces the impairment once it is present. When nimodipine treatment was continued for more than 4 months, a rapid increase in the frequency and severity of abnormal footprints was seen (Gispen et al., 1988; Van der Zee et al., 1990). The difference between nimodipine-fed and age-matched controls disappeared, not only in the footprint test but also in the sensorimotor function tests. Analysis of mortality rate revealed that life span was not influenced by long-term nimodipine treatment.

In recent years Landfield and coworkers have obtained ample evidence indicating that voltage-dependent Ca^{2+} influx is increased in hippocampal neurons of rodents during the brain aging process leading to a prolonged Ca^{2+}-activated K^+ current in hippocampal neurons called prolonged afterhyperpolarization (AHP). Results consistent with this observation have been obtained in several studies (Landfield and Morgan, 1984; Landfield and Pitler, 1984; Landfield et al., 1986; Landfield, 1988a,b). Other investigators have also found clear evidence of aging-related changes in various aspects of brain Ca^{2+} homeostasis (Gibson and Peterson, 1987; Khachaturian, 1984; Michaelis et al., 1984). The findings from Landfield on voltage-dependent Ca^{2+} currents have led to the specific hypothesis that age changes in membrane Ca^{2+} channels underlie a significant fraction of the disturbed Ca^{2+} homeostasis seen in brain aging (Landfield, 1987). A few studies support the possibility of counteracting age-related decline with magnesium, a strong Ca^{2+} antagonist. High magnesium diets were able to improve maze reversal learning in aged rats and also to improve hippocampal frequency potentiation in intact rats (Landfield and Morgan, 1984). Moreover, in hippocampal slices, elevated Mg^2: Ca^{2+} ratios strongly improved intracellular measures of frequency potentiation, particularly in aged rat slices (Landfield et al., 1986). Frequency potentiation is a hippocampal variable that exhibits maybe the most consistent pattern of age-related changes and is significantly impaired in aged animals (Landfield, 1988a); moreover, disturbances of frequency potentiation were correlated with impaired learning and memory in old rats (Landfield, 1988a). From these results it was concluded that aging affects primarily the L-like Ca^{2+} channels, and that these changes can be modulated with nimodipine (Landfield, 1989). This was observed both in intact animals and in *vitro* tests. Binding of ^{3}H-nimodipine to hippocampal tissue was reduced per milligram of protein in aged rats, compared to young rats. This reduction could reflect either a reduced inactivation of Ca^{2+} channels in aging rat brain (since binding of nimodipine occurs primarily in the inactivated state), or a loss of neurons and replacement by glial cells or processes that contain fewer Ca^{2+} channels. These data suggest that analyses

of changes in the kinetics of Ca^{2+} currents may be an important adjunct to analysis of total binding capacity, in assessing aging-related changes in brain Ca^{2+} channels (Landfield, 1989).

Another observation indicating that nimodipine may antagonize or delay some manifestations of aging comes from Disterhoft (1989), who used an associative learning paradigm in rabbits. Earlier experiments from this group had indicated that AHP is reduced during associative learning in young animals. Combining Landfield's observation mentioned above that aged neurons showed a prolonged AHP (Landfield and Pitler, 1984), they hypothesized that learning deficits that accompany the aging process might be due to a difficulty in modulation the AHP response. With eye-blink conditioning in rabbits they were able to show that indeed nimodipine enhances learning in old animals (Disterhoft al., 1989). Again, as shown also by Schuurman (see above), the effect of nimodipine was not due to increasing the alertness of the animals, nor did it influence the spontaneous blinking behavior. As this task requires an intact hippocampus, it was thought that nimodipine may act by enhancing hippocampal functioning during associative learning. As for the mechanism: although it might seem that the blockade of the Ca^{2+} channels is of the greatest importance, the authors cannot exclude that an enhanced blood supply plays a role as well. Since the test was originally developed to measure associative learning in humans (Gormezano, 1966), and as there are clear parallels between several aspects of the test in both aging rabbits and aging humans, the paradigm provides the possibility in principle to extrapolate animal data to the human.

Finally, Luiten et al. (1989) studied the brains of senescent rats (at 30 months) at the ultrastructural level. They found numerous changes, both in vascular and non-vascular morphology in old animals compared to young ones. The areas they studied were selected to represent brain substrates involved in motor function and in learning and memory. The most impressive effects of long-term nimodipine treatment were: a reduction of several age-related perivascular aberrations (large and small fibrotic deposits in the basement membrane and astrocyte endfeet thickening) and a reduction in the amount of large perivascular malformations, possibly associated with perivascular amyloidosis or an unbalanced outgrowth of myosin fibers in smooth muscle cells.

Concluding remarks

The existence of voltage-dependent channels in the nervous system has long been recognized. In recent years attention has been focused on Ca^{2+} channel antagonists, known for their prominent cardiovascular effects, that would specifically affect nervous tissue. Nimodipine is already a "classical" example of a 1,4-dihydropyridine Ca^{2+} antagonist with presumed effects on the nervous system in animal and man. Regulation of cellular Ca^{2+} homeostasis is of the utmost importance to neuronal function during development, adulthood and senescence. Under many pathological conditions increased levels of

intracellular Ca^{2+} may be part of the pathogenesis. Originally, studies with nimodipine dealt with its potential beneficial effect in the treatment and prevention of cerebrovascular lesions. Yet, recent literature, as in part reviewed here, indicates significant influences of nimodipine on the nervous system and behavior. However, not in all instances it has been demonstrated that such effects are unequivocally resulting from L-channel blockade. Further work will undoubtedly address this issue in more detail. The fundamental observations that Ca^{2+} homeostasis is severely disturbed in aged nervous tissue and that nimodipine in aged animals affects various aspects of cognitive functions is a most promising new lead in our understanding of the pathogenesis and pharmacotherapy of age-related brain and mental disorders.

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Authors' address: Prof. Dr. W. H. Gispen, Rudolf Magnus Institute, Utrecht University, Vondellaan 6, 3521 GD Utrecht, The Netherlands.