Clinical Neuropharmacology
Vol. 16, Suppl. 1, pp. S5–S11
© 1993 Raven Press, Ltd., New York

Neuronal Plasticity and Function

Willem Hendrik Gispen

Rudolf Magnus Institute, Utrecht, The Netherlands

Summary: Neuronal plasticity is a key issue in neuroscience. It is defined as the capability of the neuron to adapt to a changing internal or external environment, to previous experience or to trauma. It appears that during all phases of the individual life span in the nervous system, changes take place that relate to development, degeneration, and regeneration. Growth cones are a focus of neuronal plasticity, and current views emphasize the importance of local intracellular [Ca^{2+}] to the control of their function. Hence, outgrowth of neurites from neurons in culture may be manipulated by drugs that affect intracellular Ca^{2+} homeostasis. In the adult nervous system, much research deals with synaptic plasticity, especially with the activity-dependent changes seen after long-term potentation of hippocampal synapses. As in the growth cone, such changes involve Ca^{2+}-dependent pre- and postsynaptic processes, among which is the activation of protein kinase C. During aging, Ca^{2+} homeostasis may be slightly disturbed over a long period of time that could result in loss of function seen after a short, toxic high level of intracellular [Ca^{2+}]. In this respect, the beneficial effects of chronic treatment with the L-channel Ca^{2+}-blocker nimodipine on sensorimotor function of aged rats is discussed.
Key Words: Neuronal plasticity—Calcium—Age.

Neuronal plasticity is a key issue in neuroscience. It is defined as the capability of the neuron to adapt to a changing internal or external environment, to previous experience, or to trauma. Approximately one century ago, it was finally established that the nervous system consisted of neurons and thus was cellular rather than a continuous syncytium. During most of this century, a rather static view of the nervous system prevailed in which electrical information was thought to be processed through a fixed system of neuronal wires. Once developed and matured, no additional changes in connections took place, and following damage to the system very little, if any, repair occurred. In the last decades, however, experimental evidence was collected that underscored some of the theoretical concerns neuroscientists were having with this static view. It now appears that during all phases of the individual life span, in both the peripheral nervous system (PNS) and in the central nervous system

Address correspondence and reprint requests to Dr. W. H. Gispen at Rudolf Magnus Institute, Vondellaan 6 3521, GD Utrecht, The Netherlands.

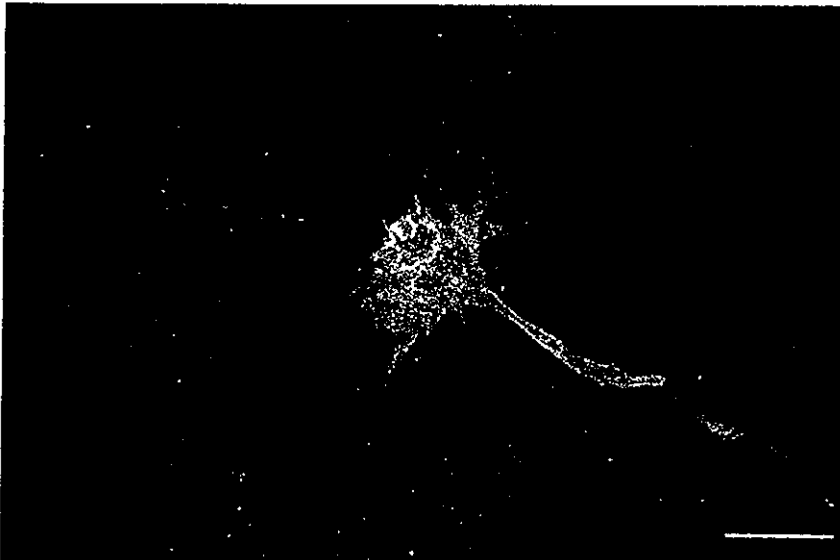

FIG. 1. B-50 immunofluorescence in a neuronal growth cone from a retinoic acid-treated P19 embryonic carcinoma culture. Reconstruction of six consecutive 1-μm thick confocal optical sections. Scale bar indicates 10 μm. (By courtesy of E. R. A. Jap Tjoen San and M. Kluytmans.)

(CNS) changes take place that relate to development, degeneration, and regeneration, providing a rather dynamic nature to the once "static" wiring system.

In 1890, the Spanish neuroscientist Ramon y Cajal first described the expanded tips of growing nerve fibers and designated them as "growth cones." These growth cones possess intrinsic mechanisms for migration and neurite elongation that respond to internal and external cues or stimuli, and appear not only during development but also during repair and, as part of ongoing remodeling, during adult life. The growth cone has been a focus of neuronal plasticity research, and current views emphasize the significance of local intracellular [Ca^{2+}] in the control of growth cone behavior (1).

One important protein that is present in growth cones is the nervous tissue-specific phosphorprotein B-50 (also called GAP43, F1, or neuromodulin) (Fig. 1). B-50 is an acid membrane-associated substrate of protein kinase C (PKC). The protein has been sequenced recently by several groups. Although its molecular mass on 11% sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis (PAGE) was estimated to be 48 kDa, the cDNA sequence predicts a 23.6-kDa protein with 226 amino acids. The sequence predicts a number of potential phosphorylation sites at which Ser^{41} is the PKC phosphosite (presumed to be the only physiologically relevant phosphorylation site). The protein binds calmodulin in vitro in the absence of calcium and has been proposed as a local calmodulin store in the nerve terminal (2).

The calmodulin binding is negatively correlated with the degree of PKC-mediated phosphorylation of B-50. As PKC is an important Ca^{2+}-activated protein kinase, it

may be that the significance of Ca^{2+} for growth and motility in part takes place via a Ca^{2+}/PKC/B-50 sequence. Interestingly, B-50 expression is developmentally regulated. It is highly expressed as soon as fetal neurons differentiate, following which the expression of the protein selectively decreases with age. In the immature neuron, the protein is highly expressed in growth cones, and in the mature neuron it is in the presynaptic axon and terminal region. Several reviews have recently discussed the potential role of this protein in growth cone function and neurite formation. Its precise function has not been delineated, but as yet its importance to neuronal plasticity seems undisputed. To what extent the Ca^{2+}- and PKC-mediated phosphorylation is essential is still under investigation (see below) (2).

NEURONAL PLASTICITY AND CALCIUM

Normally, the resting free [Ca^{2+}] within a neuron is maintained at 10^{-8}–10^{-7} *M*. Such a low free cytosolic [Ca^{2+}] allows for a rapid and efficient detection of transient, small increases in intracellular [Ca^{2+}] as a result of transmembrane signal transduction. Apparently, many stimuli that influence neuronal functions affect intracellular [Ca^{2+}] by opening voltage-sensitive or receptor-mediated, plasma membrane Ca^{2+} channels or intracellular Ca^{2+} stores. The neuron is equipped with a variety of systems that buffer the rise in intracellular [Ca^{2+}] and provide for Ca^{2+} homeostasis. Kater et al. (3) have demonstrated that action potentials and neurotransmitter action converge at the level of the membrane potential, which in large part determines growth cone intracellular [Ca^{2+}]. They speculate that their so-called Ca^{2+} hypothesis provides a model for a basic mechanism in the regulation of growth cone behavior. The significance of their hypothesis is that neurite outgrowth can only proceed when intracellular [Ca^{2+}] levels lie within a specific outgrowth-permissive range. Cessation of outgrowth can be induced by signals that elevate Ca^{2+} levels above that range. Extreme excitatory imbalance in neurotransmitter input, resulting in a marked elevation of intracellular [Ca^{2+}], may lead to cell death. An example is the study by Robson and Burgoyne (4) using cultured dorsal root ganglion cells from neonatal rats under highly favorable conditions: 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%. The drugs and K^+ cause depolarization, concurrent with ion shifts across the membrane, resulting in a high intracellular Ca^{2+} level. When the cells were treated with nifedipine, a dihydropyridine compound blocking L-type Ca^{2+} channels, the inhibition was reversed and outgrowth returned almost to normal levels.

It is apparent that Ca^{2+} homeostasis is also of significance to neuronal plasticity in the aging brain. In fact, when re-examining the role of Ca^{2+} regulation in nervous system aging, Khachaturian (5) proposed that age-related neuronal and brain deficits may originate from even a small disturbance in Ca^{2+} homeostasis that exists over a long period of time. Collectively, the data suggest that the intracellular Ca^{2+} level is essential in neuronal outgrowth, degeneration, and repair, and thus neuronal plasticity in general. This notion is the rationale for testing putative drugs that are known to affect intracellular Ca^{2+} levels in models that examine neuronal protection and re-

pair. In this respect, much attention has been given to nimodipine, a dihydropyridine that blocks Ca^{2+} channels of the L type and penetrates the brain parenchyma relatively well.

SYNAPTIC PLASTICITY

Much research focuses on the role synaptic plasticity plays in the response of the neuron to previous experience, and more precisely on how experience is acquired and stored. Most of our thinking is influenced by the hypothesis put forward by Hebb in the 1940s that the basis for information storage lies in functional modifications of the junctions between neurons, the synapses. The efficacy of communication between two neurons is related to the degree of activation and previous experience. Therefore, neuroscientists were fascinated by the observation of Bliss and Lomo in the 1970s, indicating long-term changes in synaptic efficacy following high-frequency stimulation of a given synaptic junction. This phenomenon, known as long-term potentiation (LTP), currently is considered one of the most appropriate experimental models in the study of synaptic plasticity related to information storage in the brain (6). Collingridge (see ref. 6) demonstrated that following tetanic stimulation of glutaminergic synapses in the hippocampal CA1 pyramidal cells, increased depolarization of the postsynaptic plasma membrane removes the Mg^{2+} from Ca^{2+} channels that are coupled to glutamate receptors of the N-methyl-D-aspartate (NMDA) type. Thus, the induction of LTP certainly involves a Ca^{2+}-mediated change in the postsynaptic compartment (6). We and others have studied the role of presynaptic events in LTP. Bliss and others reported that following a tetanus an enhanced release of glutamate occurred that was possibly triggered by a retrograde signal from the post- to the presynaptic site of the synapse (6). As discussed above, B-50/GAP_{43} is associated with the inner leaflet of the presynaptic membrane and is a well-characterized substrate to PKC. Activation of PKC is thought to involve initial ligand binding to the membrane and subsequent stimulation by diacylglycerol. Diacylglycerol is produced by phospholipase C-catalyzed hydrolysis of polyphosphoinositides in the membrane. The kinase can also be activated by synthetic diacylglycerol derivatives such as 1,2-dioctanoylglycerol and tumor-promoting phorbol esters.

Routtenberg (see ref. 6) has shown that LTP elicited in vivo is associated with a translocation of PKC activity to the membrane and a sustained increase in vitro of F1 phosphorylation (6). We have demonstrated that in situ B-50 phosphorylation is closely correlated with short-term effects on neurotransmitter release. Chemical depolarization induces an increase in B-50 phosphorylation that requires extracellular Ca^{2+} and can be prevented by PKC inhibitors. Phorbol esters, which increase transmitter release under depolarizing conditions, also stimulate B-50 phosphorylation. Long-term effects of phorbol esters, which resemble some features of LTP, are paralleled by a sustained increase in B-50 phosphorylation (6).

Recently, it was found that B-50 phosphorylation measured by quantitative immunoprecipitation in rat hippocampal slices incubated in the presence of radiolabeled inorganic phosphate was increased for at least 1 h after the induction of long-term potentiation in the CA1 region. No significant changes in B-50 phosphorylation were observed in untetanized slices stimulated at low frequency. The direct

demonstration of an increased phosphorylation of the protein B-50 during long-term potentiation is consistent with the hypothesis that presynaptic mechanisms contribute to the maintenance of LTP (7).

The biological significance of NMDA-mediated synaptic plasticity as seen in LTP comes from elegant studies of the group of Morris (6). These investigators studied spatial orientation in a water tank containing a rescue platform that was placed under the surface of milky water. Its location can only be remembered by making use of external cues. It is well known that in the rat the hippocampus plays an important role in the acquisition and consolidation of spatial memory tasks. By application of low doses of specific NMDA-receptors antagonists, Morris was able to show that rats so treated displayed inferior acquisition of such a task (6). In conclusion, synaptic plasticity, like growth cone plasticity, is controlled by changes in intracellular Ca^{2+} concentrations. Induction seems to depend on an NMDA-receptor–mediated Ca^{2+} influx in the postsynaptic compartment, whereas maintenance may involve a Ca^{2+}-mediated regulation of PKC activity in the presynaptic and postsynaptic compartments.

AGE-RELATED DEFICITS IN NEURONAL PLASTICITY: SENSORIMOTOR FUNCTION

Age-related deficits in neuronal function may relate to altered neuronal Ca^{2+} homeostasis (5). It is well established that aging rodents display a severe disturbance in sensorimotor functioning. Schuurman et al. (8) studied the effect of age on the ability of rats to cope with various motor tasks such as rod balancing, pole climbing, and suspended hanging. Senescent rats (24–30 months old) show a serious deterioration in these various motor function tasks. Feeding such rats with food pellets containing 250 or 860 ppm of nimodipine resulted in an improved performance in the rod-balancing test compared to the performance of vehicle-treated control aging rats, suggesting that long-term nimodipine treatment may counteract the age-related impairment of sensorimotor function.

To test the generality of that notion, Schuurman and co-workers studied the effects of aging and of nimodipine treatment on qualitative aspects of locomotion. Rats of different age categories and also old rats treated with nimodipine were subjected to a footprint test (8). Whereas young adult rats walk on their toes, 24-month-old subjects also place their heels on the ground and they turn their hindfeet in the outward direction ("exorotation"). Prints produced by rats aged 28 months or more exhibit a "fuzzy" feature. Other changes, which are probably caused by a reduction in or loss of central coordination of the movements of the hindlegs during locomotion, have been described in detail elsewhere (8). In several experiments, it was demonstrated that the onset of abnormal footprints was significantly delayed in old rats that were fed with nimodipine-containing food from the age of 24 months on. The most effective concentration of nimodipine was 860 ppm. This corresponds to a daily intake of approximately 30 mg/kg of body weight (the average daily food consumption of old rats with a body weight of 400 g was 15 g). Lower concentrations (250 and 500 ppm) were less effective and a higher concentration (1,200 ppm) was equally effective in delaying abnormal locomotion.

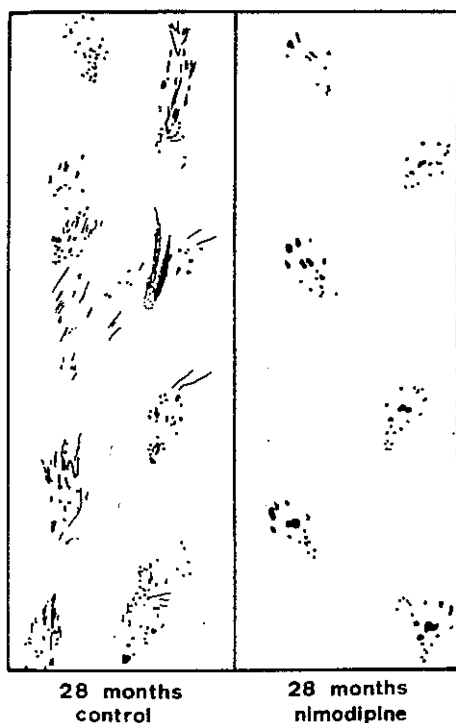

FIG. 2. Footprints of a 28-month-old rat and a similar old rat treated for 16 weeks with nimodipine-containing food (860 ppm).

In another experiment (9), it could be demonstrated that nimodipine treatment (860 ppm) of 24-month-old rats, which already showed abnormal prints of the hindlimb, results in an improvement of walking patterns, as measured 6 weeks after the onset of the experiment. Therefore, long-term nimodipine treatment not only delays the onset of abnormal walking, but also reduces the impairment once it is already present. In Fig. 2, footprints of a 28-month-old rat treated for 16 weeks with nimodipine and prints from a 28-month-old control rat are presented. When nimodipine treatment was continued after the age of 28 months (more than 4 months of treatment), a rapid increase in the frequency and severity of abnormal footprints was seen. The difference between nimodipine-fed and age-matched controls disappeared. This was not only true for the footprint test but also for sensorimotor function tests like the balance rod and pole-climbing tests. Analysis of the mortality rate revealed that the lifespan was not influenced by long-term nimodipine treatment. At the completion of the experiment, the sensory nerve conduction velocity (NCV) and motor NCV in the sciatic nerve were measured. The NCVs in placebo-treated senescent rats were lower than those obtained in young adult rats. Chronic treatment with oral nimodipine had resulted in significantly higher sciatic NCVs. Histological analysis revealed that the fiber density in the sciatic nerve of aged rats is much lower than in young adult rats and that nimodipine had significantly enhanced this density by increasing the number of fibers, compared to age-matched, placebo-treated, control rats. In a later study by De Jong et al. (10), only minor effects of chronic oral nimodipine treatment on peripheral nerve histology were obtained. In the study of Van der Zee et

al. (9), the rats were selected for poor walking performance at 24 months of age, whereas in the study by De Jong et al. (10), rats were randomly chosen at 16 months of age. Thus, the effect of nimodipine on histological parameters of the peripheral nerve appears to be greater when the age-related deficits in peripheral nerve function are pronounced.

CONCLUDING REMARKS

In this short review, I have discussed some of the aspects of neuronal plasticity in the context of neuronal function. It is clear that Ca^{2+} homeostasis and Ca^{2+}-mediated cellular responses are of significance to neuronal plasticity during development (growth cone behavior), adulthood (synaptic plasticity), and aging (functional defects). Hence, it is not surprising that drugs that influence cellular $[Ca^{2+}]$ such as nimodipine, are of potential use in neuronal repair and protection when the neuron must rely on its plastic repertoire. By supporting neuronal plasticity and function in the aging human brain, one may expect to improve the quality of brain and behavior, and thus of life, in an aging society.

REFERENCES

1. Letourneau PC, Kater SB, Macagno ER, eds. *The nerve growth cone.* New York: Raven Press, 1991.
2. Gispen WH, Nielander HB, De Graan PNE, Oestreicher AB, Schrama LH, Schotman P. Role of growth-associated protein B-50/GAP-43 in neuronal plasticity. *Mol Neurobiol* 1991;5:61–85.
3. Kater SB, Mattson MP, Cohan C, Connor J. Calcium regulation of the neural growth cone. *Trends Neurosci* 1989;11:315–21.
4. Robson SJ, Burgoyne RD. L-type channels in the regulation of neurite outgrowth from the rat dorsal root ganglion neurons in culture. *Neurosci Lett* 1989;104:110–7.
5. Khachaturian ZS. The role of calcium regulation in brain aging: reexamination of a hypothesis. *Aging* 1989;1:17–34.
6. Baudry M, Davis JL, eds. *Long term potentiation: a debate of current issues.* Cambridge, MA: MIT Press, 1991.
7. Gianotti C, Nunzi MG, Gispen WH, Corradetti R. Phosphorylation of the presynaptic protein B-50 (GAP-43) is increased during electrically induced long-term potentiation. *Neuron* 1992;8:843–8.
8. Schuurman T, Traber J. Effect of nimodipine on behavior of old rats. In: Traber J, Gispen WH, eds. *Nimodipine and central nervous system function: new vistas.* Stuttgart: Schattauer, 1989:195–208.
9. Van der Zee CEEM, Schuurman T, Gerritsen van der Hoop, Traber J, Gispen WH. Beneficial effect of nimodipine on peripheral nerve function in aged rats. *Neurobiol Aging* 1989;11:451–6.
10. De Jong GI, Jansen ASP, Horvath E, Gispen WH, Luiten PGM. Nimodipine effects on cerebral microvessels and sciatic nerve in aging rats. *Neurobiol Aging* 1991;13:73–81.