Neurotrophins: evolution of concepts on rational therapeutic approaches

ABSTRACT

Neurotrophins (NT) were “brought to life” by Rita Levi-Montalcini and Stanley Cohen, thanks to their discovery of the nerve growth factor (NGF) isolated from the malignant tumor. A hint got from the cancer studies on the presence of NTs and other molecules like metalloproteases, which control neuronal remodeling and extracellular matrix in tumors, was the stimulus to investigate the role of these molecules in physiology and post-lesion plasticity of the nervous system. NTs have long been identified as drivers of neurogenesis during development and regeneration of the nervous system. Thousands of investigations spanned from early sixties till now provided strong evidence that this small family of molecules is indispensable for central and peripheral nervous system maintenance throughout the whole life of different animal species. In mammals NTs regulate sensation, movement, behavior, and cognition. A problem, which accompanies the majority of experimental therapies using NTs following injury of the nervous system, stems from unspacific and uncontrolled stimulation of the whole circuitry of preserved neurons. Among the already identified causes of clinical trial failure with the use of NT therapy in the treatment of neurodegenerative diseases are: (1) disregarding neuronal preferences to selected NTs; (2) poor knowledge on NT pharmacokinetics (3) uncontrolled spread of NTs when administered systemically, which could have unpredictable effects on other than intended neuronal populations (4) limitations of tissue penetration of some NTs (5) disturbances of the balance between signal transmission through their Trk and LGNTR receptors. I present our contribution to the field and efforts to control spatially and temporally postinjury NT signaling.

INTRODUCTION

Where did my interest in neurotrophic factors come from? My first steps in research were accompanied by the discoveries that after damage to the nervous tissue, in the areas that are wounded and in their proximity, trophic activity of yet unidentified factors is increased [1-5]. This phenomenon, demonstrated and most extensively documented in the cerebral cortex after its damage [1,5,6] and in the hippocampus partially denervated by transection of inputs from the septal nuclei [7-9], suggested that the injured nervous system triggers not only defensive mechanisms but also the processes of active regeneration of damaged connections. Extracts obtained from wound tissue stimulated neuritogenesis, axonal growth and phenotypic maturation of nerve cells in vitro [10,11]. The results of these studies indicated that unidentified, soluble substances, present in brain extracts, can counteract the effects of damage by weakening neurodegeneration and/or stimulating regeneration processes. If we assume that an increase in trophic activity in the denervated tissue is a reaction to the interruption of signals from damaged nerve tracts, it can be hypothesized that the lesion causes changes that reflect to some extent developmental program, when the target structure is “preparing” for nesting of the ingrowing neuronal fibers by sending trophic signals. It was a very attractive hypothesis.

Assuming that the survival of neurons in the mature brain depends on trophic factors to similar extent as during development, the view was formulated that damaged neurons die due to insufficient supply of trophic factors [12-14]. This would happen, despite initiation of fiber growth in response to molecular cues involving trophic factors present in the extracellular milieu, if tissue concentration of these factors was insufficient to maintain the damaged neuron and to stimulate repair processes. Also, isolation of target structures by damage would render trophic factors unable to reach neurons.

THE BEGINNING OF EXPERIMENTAL THERAPIES WITH NEUROTROPHINS

The earliest study undertaken to counteract the presumed deficits of trophic factors in damaged brain was on the efficacy of the administered nerve growth factor (NGF) in postlesion recovery of the cholinergic system. NGF is a prototype neurotrophin (NT), that Rita Levi-Montalcini and Stanley Cohen discovered and
characterized in the 1950s, demonstrating its ability to stimulate growth of sensory and sympathetic neurons towards sarcoma tissue rich in that factor. Research with the use of NGF was also undertaken at my parent Laboratory of Neurochemistry at the Nencki Institute. Our first experiments dealing with NGF were focused on the impact of NGF administration on changes caused by destruction of pathways running from the septum to the hippocampus, which leads to partial denervation of the hippocampal formation and retrograde degeneration of the septal neurons. The other approach was to destroy or damage target areas of investigated projections. An example is partial cortical devascularization, a model of permanent cortical stroke with contribution of subarachnoid extravasation. It leads to slow, retrograde degeneration of cholinergic projection derived from the forebrain nucleus basalis magnocellularis (nbm) neurons, which are located subcortically. It is combined with progressive degeneration of cortical neurons in the ischemic area and the loss of brain tissue in the ischemic core, which in turn causes degeneration of cortico-thalamic and thalamo-cortical projections. In considering the limited self-healing ability and searching for therapy, it was assumed that an increase in the level of trophic factors in wounded tissues may make those neurons that have retained some of the connections to the target structures not perish; only cells that are seriously damaged are lost. This would create a chance to weaken neurodegenerative changes and stimulate the regeneration of neurons by supplementation with trophic factors. The most frequent way of NT application at that time was its intraventricular infusion via minipump, implanted at the animal’s back. Several research groups, almost simultaneously, have attempted to verify these possibilities.

NGF proved to be effective in preventing atrophy of cholinergic neurons after both types of damage. It also counteracted the decreased level and activity of ChAT and AChE cholinergic enzymes in the hippocampus and cerebral cortex [12,15-17]. Claudio Cuello’s group also showed that in the model of partial, cortical devascularization, exogenous NGF stimulates synaptogenesis and induces hypertrophy of cholinergic terminals in the preserved parts of the cerebral ischemic area and the loss of brain tissue in the ischemic core, which in turn causes degeneration of cortico-thalamic and thalamo-cortical projections. In considering the limited self-healing ability and searching for therapy, it was assumed that an increase in the level of trophic factors in wounded tissues may make those neurons that have retained some of the connections to the target structures not perish; only cells that are seriously damaged are lost. This would create a chance to weaken neurodegenerative changes and stimulate the regeneration of neurons by supplementation with trophic factors. The most frequent way of NT application at that time was its intraventricular infusion via minipump, implanted at the animal’s back. Several research groups, almost simultaneously, have attempted to verify these possibilities.

Those works, showing the beneficial effects of trophic factors on repair processes and providing the approaches to address questions about NT receptors localization and properties, were the impulse for my projects undertaken during my postdoctoral studies.

Meeting Claudio Cuello, thanks to the scientific contacts of Barbara Oderfeld-Nowak, my Promoter, at the Congress organized by our Group at the Nencki Institute in 1989, was an opportunity to establish cooperation with this specialist in the field of regenerative pharmacology. Professor Cuello, a close associate of Cesar Milstein, who was a discoverer of the principle for production of monoclonal antibodies and the Nobel Prize winner, was a co-discoverer of bispecific hybrid antibodies [22] and the recognized authority in immunohistochemistry. Leaving for post-doctoral training at his laboratory at the McGill University, I had an opportunity to master that technique, very new at that time, and use it to answer questions on the effectiveness and mechanisms of activity of neurotrophic factors in the brain after damage. We aimed to deepen the analysis of changes occurring after both types of damage by enriching that line of research with new aspects: the possibilities for repair of the brain cholinergic neurons with brain derived neurotrophic factor (BDNF) and neurotrophin 3 and 4 (NT-3 and NT-4), and the cellular mechanisms underlying NT effects. As already mentioned, at that time, high-affinity NT receptors have been just discovered, no signaling pathways were identified, and basic questions on their tissue distribution and regulation, crucial for therapeutical targeting, were not answered yet.

HIGH- AND LOW-AFFINITY NGF RECEPTORS: NBM CHOLINERGIC NEURONS RESPONSE TO CORTICAL DEGENERATION AND MODULATORY EFFECT OF EXOGENOUS NGF

With the use of cortical devascularization model, postlesion changes can be tracked simultaneously in the cerebral cortex, nbm and the thalamic nuclei. This renders valuable possibility to simultaneously test sensitivity of several classes of neurons characterized by different degree of damage. Also, it allows to examine their molecular milieu, in which processes progressing after damage occur at different rates and with varying intensity. Due to the fact that exogenous NGF was effective in counteracting atrophy and dysfunction of nbm cholinergic neurons [18,23], we were interested in whether administration of NGF can increase its tissue level to the extent, which would activate (and not desensitize) the pool of NGF receptors expressed in nbm neurons. Such assumption was based on results showing that NGF causes regulation of mRNA transcription of NGF receptors in newborn rats [24]. The explanation of whether NTs administered to adult animals with brain damage cause upregulation of their high-affinity Trk receptors, and determination of changes in receptor expression at different time after injury, would allow to characterize potential changes in neuron receptivity to NGF, and to plan optimal experimental therapy. We have investigated whether the damage alters the expression of high affinity NGF receptors (TrkA) and low affinity neurotrophin receptors (LNTR-p75) at different time after the extravasation. And if it does, whether and how NGF administration modifies their expression, which we analyzed by in situ hybridization. It turned out that the reinnervation of the cortex is accompanied by changes in the expression of both NGF receptors in nbm neurons [25]. An initial decrease in TrkA receptor mRNA was accompanied by a short-term increase in the LNTR-p75 receptor mRNA level and followed by its decline in later postlesion period [26]. Today we know that lowering LNTR-p75 receptor expression may reduce signal transmission mediated also by other NTs; later studies proved that LNTR-p75 is common to all NTs and can participate in signaling pathway which leads to inhibition of axonal growth.
Administration of NGF caused an increase in the expression of both types of NGF receptors in nbm neurons. What’s more, already in the first week after the lesion, NGF compensated for the decrease in the TrkA receptor mRNA level, and two weeks after (one week after discontinuing NGF), a further increase in TrkA mRNA exceeded the control level. Administration of NGF caused even faster regulation of LNTR-p75 receptor mRNA transcription, increasing its expression above control values already in the first days after surgery, but in contrast, no effect on LNTR-p75 was found after one week of discontinuing NGF administration.

The obtained data showed that exogenous NGF may induce rapid regulation of both receptors at the transcription level and cause long-term stimulation of TrkA receptor expression by this factor [26]. The latter result had a practical significance, as it suggested that even short-term administration of NGF may result in increased neuronal receptivity beyond the “therapy” period and more efficient induction of intracellular processes. The effect of exogenous NGF on the expression of TrkA receptors was also reported by studies of receptor responses in axotomized septal cholinergic neurons [27].

Searching for the mechanisms of NGF activity we asked whether NGF administration modifies expression of growth associated protein 43 (GAP-43) mRNA in nbm neurons and brain neurons [26]. The result did not confirm our hypothesis that one of the ways NGF affects the formation of synaptic terminals in the cerebral cortex, is by stimulation of GAP-43 expression, without ruling out the possibility that NGF regulation of GAP-43 occurs at the translational level. The question remained, which factors regulate the expression of GAP-43 in damaged nerve tissue. Today we know that GAP-43 is regulated by NT-3 and NT-4, indicating the involvement of synergistic/additive NT signaling in the reorganization of neuronal networks after damage [28].

**THE MECHANISM OF ACTION OF aFGF ON NBM NEURONS AFTER DEVASCULARIZATION: ARE ASTROCYTES INVOLVED?**

The effectiveness of aFGF in counteracting degeneration of nbm neurons [29] in the absence of FGFR receptors on these cells [30,31] indicated that the effect is indirect, driving our attention towards possibility of contribution of non-neuronal cells in the formation of “cellular neurotrophic loop”. We have conducted experiments to discover the pathway on which aFGF supports the damaged nbm neurons [25]. Based on individual reports indicating that aFGF stimulates the expression of NGF in astrocytes in vitro [32, 33], we hypothesized that aFGF administered systemically increases the level of endogenous NGF pools in consequence of activation of NGF transcription/translation in the preserved regions of the cerebral cortex, involving non-neuronal cells.

We showed that aFGF, administered intracerebroventricularly, caused a significant, several fold increase in the level of NGF mRNA and protein in the cerebral cortex [25]. This confirmation of our hypothesis pointed to one of the possible ways of modulation of cholinergic neurons by aFGF, by increasing the level of endogenous NGF pools. However, even more important outcome of the study was an observation of NGF increase in the hemisphere contralateral to the injured one [25]. The latter effect was an undesirable consequence of the aFGF administration and called for search of more selective ways of trophic factors administration and supply.

In the experiments described above we also observed a spontaneous increase in the level of NGF protein in the cerebral cortex on the injured side [25]. We suspected that this increase is due to disturbance of NGF axonal transport from target regions, as described later on for TrkA and LNTR receptors in damaged septo-hippocampal pathways [34]. The second possibility was that an increase in NGF level was the result of increased synthesis of this protein in cortical cells.

In 1991, Bakhit et al. [35] published a study in which the phenomenon of elevation of glia-derived NGF was described for the first time, as a result of destruction of hippocampal neurons. Subsequent work, including our experiments, showed that hippocampal damage with strong glial activation leads to the expression of NGF and TrkA receptors in reactive astrocytes [36,37]. Is the hippocampal formation trophically privileged, what would explain vigorous development of reactive synaptogenesis, collateral sprouting and reorganization of connections following damage or denervation in this structure [7,8,18]? Earlier works of Nieto-Sampedro et al. (1982) and of Whittemore et al. (1985), but also our results [25] pointed to similar possibility in the cortex of the brain. We have attempted to find an answer to the question as to whether the spontaneous increase in the level of NGF protein in the cortex on the injured side may result from an increase in NGF levels in glial cells.

This concept resulted in studies in which we demonstrated, using immunocytochemistry, that the increase of NGF protein expression in the preserved regions of the cortex occurs not only in neurons but also in non-neuronal cells. An increase in NGF was spread throughout cerebral cortex involving temporal and the occipital cortex, documenting for the first time the expression of NGF and its receptor in a population of cortical reactive astrocytes. It also confirmed data published by other researchers and by our team, indicating that after damage to the brain tissue, reactive astrocytes can take up the synthesis and respond to trophic proteins [37,38]. These data may also be an indication that the effect of aFGF on NGF expression and the attenuating effect of this factor on neurodegeneration may result from the recruitment of a certain pool of cortical astrocytes to the molecular program of NT synthesis. Due to the fact that astrocytes constitute large, strongly reactive population of central nervous system cells, manipulating their activity may be a powerful tool modifying the function of nerve cells. Our further studies widened our understanding of the scope of astroglial neurotrophic responses, showing the phenomenon of NGF and TrkA receptor induction in astrocytes of the thalamic nuclei in response to cortical damage. We have found that the induction of NT proteins in astrocytes is closely related spatially and temporally to the progressive degeneration and denervation of dorsolateral (DL) and ventromedoral (VPL, VL) nuclei under the influence of
cortical damage. We have also shown that this induction occurs in the astroglial cells, which become reactive. This result again pointed to the important role of activated glial cells in the trophic function of damaged nerve tissue.

**ATTEMPTS TO COUNTERACT DEGENERATION OF NBM NEURONS WITH BDNF AND NT-3**

In the early 1990s, brain derived neurotrophic factor (BDNF), discovered in 1982, was already shown to be a NT produced by many more central nervous system structures than NGF. BDNF was detected primarily in the neurons of the hippocampus, cerebral cortex and olfactory bulb, but also in retinal neurons, cerebellar granular cells and brainstem nuclei [39-42]. BDNF is detected in numerous neurons and fibers that reach the spinal cord [43-45]. Despite the prevalence of this protein, little was known about the BDNF function. In 1990, Alderson and colleagues showed in vitro that not only NGF, but also BDNF affects the survival of basal ganglia cholinergic neurons. It was a reason for us to undertake research to determine whether the nbm cholinergic neurons are sensitive to BDNF also in vivo and whether the nbm cholinergic neurons are sensitive to the newly discovered NT-3. Target neurons for NT-3 in the central nervous system were not yet identified at the time of our research. Evaluation of changes was carried out by morphometry of cholinergic neurons labeled with antibodies recognizing the ChAT, a reliable marker of cholinergic neurons. It was also examined whether BDNF and NT-3 alter the expression of the LNTR receptor in nbm neurons. We showed that none of the tested NTs counteracted dystrophy of nbm neurons and reduced degeneration of cholinergic fibers [46]. None of the factors influenced LNTR receptor immunoreactivity either, suggesting that the two compounds do not affect the level of the LNTR receptor protein [46]. The obtained results suggested a lower sensitivity of nbm neurons to BDNF than to NGF and their insensitivity to NT-3. That result was confirmed at the same time for the population of cholinergic septal neurons in studies of other research groups. In parallel to our data, other reports [47] drew attention to the very limited penetration of BDNF by brain tissue. BDNF, in contrast to NGF, when administered intraventricularly, did not penetrate brain parenchyma, accumulating around brain ventricles. That conundrum found its explanation in the discovery of TrkB and TrkC truncated forms (TrkTK) of receptors. These forms, TrkBTK in particular, are considered to be “molecular sponges”, possibly protecting NTs from dispersion from the place they are released, and providing a mechanism for selective targeting. This was a very important observation indicating the need to optimize and search for alternative ways of administering BDNF or increasing the tissue level of this NT.

The neuroprotective effects of NTs demonstrated after the majority of experimental trials after nervous system trauma [16,48-50], as well as after ischemic lesions [23,51], arose great hopes for the therapeutic use of these proteins in the repair of the nervous system in humans. Clinicians have attempted to administer NGF to patients with Alzheimer’s disease. Concurrently, experimental work, including our research discussed earlier, made it possible to learn on the mechanisms of action of these compounds [25,26], but also revealed limitations in their operation and unwanted effects of therapy (discussed in: [52]). Attempts to administer trophic factors to patients with Alzheimer disease and Parkinson disease did not bring the expected results and discouraged clinicians to use NTs as potential therapeutic agents. The effects of clinical trials and prospects of NT therapy were presented comprehensively in our review [53]. Among the already identified causes of clinical trial failure with the use of NT therapy in treatment of neurodegenerative diseases, the following should be mentioned: (1) disregarding neuronal preferences to selected NTs; (2) poor knowledge on pharmacokinetics of neurotrophins; (3) uncontrolled spread of NTs when administered systemically, which could have unpredictable effects in their operation on other than intended neuronal populations; (4) limitations of tissue penetration of some NTs (5) disturbances of the balance between the signal transmission system through Trk and LNGTR receptors.

While the reasons for failures should be attributed to insufficient knowledge about NTs and their receptors in early 1990s when clinical trials were undertaken, only the first two reasons could be avoided if systemic administration of these substances was to be maintained [46,53]. These limitations provoked the attempts to seek for alternative methods to increase the level of NTs and their receptors in the nervous system.

We and others showed that brain injury alone may be a stimulus to an increase of NGF expression and of TrkA in neurons and astroglial cells [25,37,54]. That slumbered potential of denervated tissue underlined the concept to look for methods to stimulate an increase of endogenous NTs above the threshold values, necessary to promote regeneration. Numerous works that appeared in the middle of 1990s on the mechanisms regulating the level of BDNF indicated that the approach, that could result in increased expression of this protein and lead to its synthesis, is the use of stimuli that stimulate neuronal activity. The questions we wanted to answer were: (1) can we modulate the level of endogenous NTs locally? (2) whether the induced changes in the level of NTs would be accompanied by appropriate changes in the expression of their Trk receptors (i.e. those which determine the beneficial effects of NTs)? (3) does modulation occur only in neurons or recruits also glial cells? (4) do experimentally induced changes in the level of endogenous NTs affect all NTs, or they are selective?

After return from postdoctoral training, I made the decision to continue the NT thread of my research.

**ATTEMPTS TO STIMULATE THE EXPRESSION OF NTS AND THEIR RECEPTORS**

The starting points for our research were the results indicating that BDNF is that NT whose share in the activity and plasticity of numerous neural populations seems dominant. Neuronal activity and high frequency electrical stimulation increase the expression of BDNF at both transcript and protein level [55]. These relationships are mutual because NTs are involved in regulation of neuronal activity [56-61]. In ex vivo studies on brain sections, in conditions of BDNF
deficiency or blockade of TrkB receptor mediated signaling, long-term synaptic potentiation (LTP; an electrophysiologica
cal phenomenon which can enhance synaptic transmission and
reflect neural mechanism for information storage), cannot
be induced. In addition, a reduction in the BDNF level
(to the one found in BDNF deficient heterozygous mutants)
is sufficient to prevent LTP induction in the rat hippocampal
tissue sections. Importantly, this can be counteracted by
administering exogenous BDNF [57,62]. In 1995, the activa-
tion of the nervous system caused by physical exercise was
found to be an important stimulus which increased BDNF
and NGF transcripts in the rat brain [63]. The next work of
Cotman’s group confirmed these observations and proved that
the level of NT expression is correlated positively with the
intensity of training [64]. In the light of those data, it
seemed likely that other groups of neurons whose activity is
regulated by BDNF may respond in the same way to stimu-
lation through training. If this approach proved to be effect-
ive, it could be an attractive method of stimulating recovery
processes. In particular, it could be used in sustaining
neurotrophic function after spinal cord injury, where one of
the conditions of therapeutic success is to maintain the ac-
tivity of neural networks below the lesion site.

The possibilities of counteracting neurodegeneration and
stimulation of repair processes after spinal cord injuries are
the subject of our research conducted over the last twenty
years. Our experiments have shown that in the rat’s spinal
cord there are numerous groups of neurons equipped with
TrkB receptors and containing BDNF; in particular, spinal
cord motoneurons (MNs) which transmit signals to the
muscles to drive locomotion, contain BDNF, highly ex-
pressed in their dendritic compartment [44,45]. Based on
these and previously described data, we have formulated
hypothesis that long-term locomotor training should result in
an increase in the expression of BDNF and its TrkB recep-
tor in the spinal cord. In order to investigate this possibil-
ity, we conducted a study on the impact of training on the
location and level of BDNF and TrkB receptor expression
in the spinal cord. To gain an opinion on whether move-
ment training can stimulate various neurotrophic systems,
we also investigated its effect on the expression of NT-4 and
NT-3 proteins as well as TrkA and TrkC receptors.

The results of the research proved the validity of our hy-
pothesis. We have shown that moderate, long-lasting loco-
motor training in rats not only increases BDNF level [45,65],
but also stimulates the expression of NT-4 in the spinal cord
[45]. As a result of exercise the level of NTs can be increased
in cells belonging to different populations. Also, the training
clearly increases both the number of immunocytochemi-
cally detected cells equipped with the TrkB receptor and the
intensity of TrkB labeling in these cells. This effect was the
strongest in the small cell population identified as oligoden-
droglial cells that surround the large neurons in lamina IX
[44,45]. We were the first to show that oligodendrocytes can
receive neurotrophic stimuli. Our observation also suggests that
the receptivity of oligodendrocytes to BDNF and NT-4
may increase in response to training. This result shed new
light on the role of oligodendrocytes in the regulation of
nervous tissue activity. On the one hand, it may indicate the
potential of BDNF or NT-4 in stimulating the myelinating
function of oligodendrocytes, also in re-myelination. On the
other hand it may underline a new mechanism of neuron-
oligodendrocyte interaction, particularly interesting in view
of the presence of synapses on oligodendrocytes [66].

In the further research we revealed selectivity of the
training: its effect is limited to BDNF/NT-4 and their TrkB
receptor, while the NT3 /TrkC and NGF/TrkA systems re-
stained unchanged. In addition, the truncated form of the
TrkB receptor, contributing to BDNF sequestration and lim-
itiation of its dispersion in the cellular milieu [67,68], is not
regulated as a result of training of non-operated rats [45].
This result is particularly important clinically because it in-
icates the possibility of selective and local modulation of the
level of NTs and their receptors in the nervous system by
means of easily accessible physiotherapeutic methods.

The availability of BDNF changes after spinal cord injury
as a function of time and distance from the site of injury
[69,70]. Also, the expression of receptors and the intracellular
fate of NT receptor proteins undergo profound changes
after injury [25,34,70]. In particular, the LNTR NT receptor
response should become the subject of careful analysis, due
to the postulated ambivalent function of this receptor in
regulating cell survival.

Elucidating these issues may call for adaptation of train-
ing schedules and intensity, and their combination with
pharmacological approaches, but the effectiveness and
specificity of the stimuli triggered by locomotor training
makes this approach a basic non-invasive therapeutic tool in
attempts to repair damaged nerve connections and maintain
the function of denervated brain and spinal cord regions.

NEUROTROPHINS AND NEURONAL EXCITABILITY:
A RISK IN SPINAL CORD INJURY TREATMENTS?

Spinal cord injuries lead to a combination of sensory, mo-
tor and autonomic impairments, which are accompanied by
extensive reorganization of neuronal circuit caudal to the
injury. Such reorganization demonstrates the potential of
impaired circuit to undergo structural and biochemical
plasticity, as shown by the others in cats and rats [71-77]
and in a series of our studies in a model of complete spinal
cord transection (SCT) in rats [78-80]. However, changes in
cellular milieu caused by loss of descending pathways and
movement-related sensory inputs, causing altered drive to
motoneurons (MNs), prone MNs and their inputs to molec-
ular changes which result in functionally adaptive but also
maladaptive properties altering their excitability. The syn-
aptic changes on MNs and molecular mechanisms induced
in MNs associated with such plasticity are only sparsely
known.

The state of areflexia and muscle weakness that immedi-
ately follows spinal cord injury (SCI) contributes to the “spi-
nal shock” which differs in duration and severity between
species, lasting up to 2 weeks in the rat and several weeks
in humans [81]. It is gradually replaced by the recovery of
neuronal and network excitability, leading to both improve-
ments in residual motor function but also the development of
spasticity. In humans, in the months following SCI, 70-
80% of individuals develop spasticity [82,83]. It is characterized by involuntary muscle activity such as spasms, hyperreflexia and clonus [84-86].

Human studies and works on several animal models of SCI, where no clear spasticity develops, reported that both in humans and animals neurons and neuronal circuits increase their excitability and decrease their inhibitory capacity to compensate for the loss of inputs [reviewed in: [87]]. We and others contributed to these investigations showing that at 5 weeks after SCT there is a decrease in markers of excitatory and inhibitory neurotransmission in premotor interneurons below the site of transection, accompanied by a decrease in expression of KCC2 chloride ions extruder, which leads to increased MN excitability [80,88]. Thus the central question to pose is whether increased MN excitability is beneficial or detrimental for regaining impaired functions and - in this context - whether attempts to compensate deficits of innervation by supply of NTs, which maintain connections and promote plasticity is beneficial.

Classical reports [89] and [90,91] discussed that although spasticity can interfere with residual motor function and produce pain, it can also be useful, as involuntary muscle spasms temporarily increasing tone in extensor muscles can facilitate walking and standing. Therefore, finding ways to functionally reactivate the spinal cord to normalize neuronal and circuit function has been a generally accepted approach. In this way, improvements in both residual motor control and/or reductions in spasticity can occur without the unwanted side effects which are noted in clinical practice when antispastic medications are applied.

Among concepts useful in developing rehabilitative strategies to enhance recovery of posture and locomotion following spinal cord injury, exposure to the afferent and intraspinal activation patterns that are associated with standing and stepping was extensively investigated. When appropriate sensory stimuli associated with weight bearing and hindlimb activity are provided repeatedly, spinal animals can partly reacquire stepping or standing [92-99]. Because a number of data suggested that changes in the hindlimb muscles do not account for this recovery [100-103], it has been proposed that the plasticity must reside within the spinal cord [73].

Procedures based on activation addressed to the preserved network can induce plasticity within the segments caudal to lesion both in animals and in humans [75,104]. As already mentioned, locomotor training after a SCT in adult cats [73,105] and adult rats [78,79,106] results in partial recovery of gait. However, a spinal rat cannot reacquire stepping and cannot stand at all without sensory stimulation of the tail [78,79,106]. Step training in rats with SCT at a neonatal stage results in pronounced changes in the functional properties of the spinal cord circuitry, including a training-induced increase in the synaptic activation of MNs by primary afferents or intramedullary white matter tract stimulation [107]. Importantly, in conditions of neonatal SCT, locomotor training improved bipedal stepping which was accompanied by synaptic changes maintaining normal inhibitory influence on both α- and γ-MNs [77], the latter modulating excitability of α-MNs [108,109]. There is strong evidence that the potential of locomotion-derived activation of the spinal network may be related to increased NTs signaling. As described earlier, we provided strong evidence that the expression of BDNF and its receptor TrkB are stimulated by locomotor training, shown for different groups of neurons operating in the spinal network [45,65,110]. Because both BDNF and NT-3 influence the establishment of neural networks in development and regeneration, and NT-3 derived from muscle spindles regulates the synaptic connectivity between muscle sensory and motor neurons, supporting proprioceptive afferents [111], they have been postulated to be important players among factors which induce mechanisms underlying the improvement of motor abilities after SCI.

Delivery of BDNF or its gene via AAV vectors by injecting spinal cord brought some success in adult rats with spinal cord injury, leading to improvement of stepping behavior, significantly reduced deficits in neurotransmission-related proteins, normalization of CREB and synapsin in spinal neurons [80,112,113]. Our recent results indicated also that BDNF overexpressed in the spinal cord after SCT leads, within 2 weeks postlesion, to up-regulation of expression of NT-3 receptor, TrkC, in muscles. This effect suggests an increased muscle sensitivity to NT-3 signaling and better maintenance of muscle spindles. However, the use of viral vectors which cause long-term overexpression of a transgene, like BDNF, disclosed its limitations. After long-term overexpression of BDNF, symptoms of hyperexcitability of hindlimb muscles developed [80,112]. The drawback of such approach is uncontrolled expression of transgenic protein. For example, in our study, the satisfactory spatial wide-range AAV transduction of spinal neurons achieved by intraspinal delivery of AAV-BDNF construct, led to BDNF increase one hundred fold above the control levels. An interesting alternative is the mifepristone (Mfp)-regulated Gene Switch (GS) system introduced via AAV vectors, which can be used successfully in the brain, however none of these approaches was used to specifically target a neuronal network of the spinal cord [114].

Because in the meantime a picture emerged showing that a response of hindlimb extensor and flexor muscles to SCI and exercise [115] and a decrease of inputs to MNs innervating the ankle extensor and flexor muscles [79,116] are different, depending on the muscle type, a need to search for more specific, targeted approaches emerged.

HOW CLOSE ARE WE TO CURING SPINAL CORD WITH NTS?

A problem, which accompanies the majority of experimental therapies implemented following SCT, including NT treatment, stems from unspecific and uncontrolled stimulation of the whole circuitry of preserved neurons caudal to the transection site. While the paradigms of activation of the entire network lead to moderate improvement of motor functions, they do not restore functional equilibrium between different groups of MNs and muscles. These paradigms do not take into account demands of functionally different MN groups for stimulation. We provided evidence of
that imbalance, detected within several weeks after injury. As opposed to peripheral and interneuron-derived inputs to MNs innervating the ankle flexor muscle (Tibialis anterior, TA), inputs to MNs innervating the extensor muscles (Soleus, Sol and its synergist Gastrocnemius lateralis, GL) in the rat are severely impoverished after SCT at that time [79]. Namely, a profound decrease of glutamatergic proprioceptive inputs from Group I afferents and of cholinergic inputs derived from V0C modulatory interneurons was observed in Sol but not TA MNs. Group I and II sensory afferents from muscle spindles to the spinal cord play a major role in regulating spinal motor circuitry organization and output, especially after CNS injury [117,118]. Therefore, loss of Ia input on MNs and possibly on cholinergic interneurons, disturbs positive feedback provided by them about muscle contractions. It impacts also a modulatory cholinergic input to MNs, both shown to be preferentially impaired in the extensor MNs. This imbalance is associated with postsynaptic muscarinic M2 cholinergic receptor response, which changes the excitatory state of MNs. Postlesion changes in the properties and function of muscles may be related to intrinsic changes in MNs such as altered ion channels, serotonergic and muscarinic receptor and transporter concentrations in the membranes [80,88,119,120]. Again, some of these changes are clearly differentiated between extensor and flexor groups of MNs [106,120,121]. Such molecular and functional disequilibrium between different groups of MNs call for selective procedures to balance the activity of antagonistic muscles and achieve recovery of functions.

As already mentioned, we have proposed experiments to enhance synaptic Ia proprioceptive input to the motoneurons innervating the ankle extensor muscles which were found to be particularly vulnerable to the spinal cord transection [79,122]. One week of continuous burst of low-threshold proprioceptive stimulation of the tibial nerve effectively enriched both direct glutamatergic and indirect cholinergic innervation in LG α-motoneurons in the rat with intact spinal cord [122] indicating that this stimulation may be a useful therapeutic method to enhance excitatory inputs to selected group of α-motoneurons. Importantly, it also clearly increased NT-3 protein level both in the L3-L6 segments of the spinal cord and in the Sol muscle confirming an importance of this NT in the proprioceptive signaling [123,124]. However, the same strategy, when used to counteract a reduction of movement-related proprioceptive input to MNs of unloaded ankle extensor muscles in spinal paraplegic animals, did not bring an effect on the number and volume of glutamatergic and cholinergic boutons opposing LG motoneurons [125].

None of the described approaches brought about persistent, long-term improvement of motor functions in models of complete SCT. Our recent concept is to use a novel, gene transfer based approach, which maintains the possibility to selectively address treatment to the ankle extensor MNs. It is based on selective sensitization of target neurons to external stimuli by enrichment of their receptor repertoire with the use of gene transfer. This manipulation and a choice of receptors to be expressed is aimed to compare the effects caused by intermittent stimulation with external ligands, in a manner resembling electrical stimulation, and those caused by continuous tuning, exerted by endogenous BDNF. For the second purpose, BDNF/TrkB signaling pathway, enhanced by TrkB overexpression is a good choice, as BDNF was documented to protect MN from atrophy, increase MN dendritic plasticity, upregulate proteins related to neurotransmission efficiency, stimulate neuronal repair and elicit locomotion in spinal animals [80,112,126]. The virtue of this treatment is that it is based on the physiologically relevant modulation of MN activity with endogenously produced BDNF. The proof-of-concept study was published recently [127]. Until now that is the only investigation which shows that gene therapy using intramuscular (intraleural in this case) delivery of AAV-TrkB to phrenic MNs is sufficient to promote recovery of diaphragm activity and improve impaired respiratory function after spinal cord hemisection at the cervical level. The choice of this approach stems also from our preliminary observations that BDNF, overexpressed in the spinal cord after SCT, maintains integrity of neuromuscular junctions and affects muscle NT-3/TrkC neurotrophin system, leading to an increase of TrkC expression in hindlimb muscles (Głowacka A., Ji B., unpublished). We hypothesize that the gene constructs targeted to a population of extensor MNs to increase MN responsiveness to BDNF via TrkB receptors, will be expressed and promote the maintenance and restoration of connectivity of impaired projections, restore intrinsic properties of MNs and motor functions following SCT. These structural and molecular changes should lead to progressing recovery of equilibrium in innervation and signaling between MNs controlling the extensor and flexor muscles acting at the ankle joint. One of our further goals is to verify, whether this experimental paradigm secures TrkB receptors from downregulation which develops when BDNF is overexpressed in the spinal cord (see discussion in: [80]).

An approach takes advantage of the skeletal muscle and motor neurons anatomical relationship, which provides an opportunity to administer intramuscularly viral vectors containing the gene sequence for a therapeutic transgene, avoiding direct spinal cord injections and accompanying damage, which is common gene delivery approach when intraspinal transgene expression is planned [80,112,128]. It is important to limit the distribution of the transgene to only one cellular component of the spinal cord as the ubiquitous expression of a therapeutic transgene could produce unwanted effects. As already described, we and others have shown, that permanent BDNF or NT-3 overexpression in multiple neurons and/or astrocytes cause muscle hyperexcitability and spasticity [80,112,129]. It has been well documented that viral vectors such as lentiviral vectors and AAV vectors can be administered to skeletal muscle for retrograde transport along the peripheral nerve and restrict transgene expression into spinal cord or brainstem motor neurons (see in: [130]).

THE OTHER FACE OF NEUROTROPHIN SIGNALING

But the history of the role of NTs in tumors circled back [131]. Nerve outgrowth in the tumor microenvironment has recently been shown to be essential for cancer progression. The concept of nerve dependence is emerging in oncology. Recent findings have unraveled that NGF released by cancer
cells is also a driver of tumor-derived stimulation of NGF receptors on nerve endings. NT-activated signaling pathways via a family of tyrosine kinase receptors, are important for a variety of cancers and their metastatic properties. This axis is important for brain and spinal cord development efforts, ranging from pain management to neurodegeneration. Indeed, TrkA, the prototype of the NT receptor family, was first identified as part of a fusion oncogene. Moreover, Trks are widely expressed in many different organs where their misactivation has been associated with tumor formation. In consequence, nerve fibers growing into tumor microenvironment secrete neurotransmitters, which can stimulate both the growth of tumor cells and angiogenesis. This trophic role of NGF and other NTs in cancer, which is beyond the scope of my research, was proposed to be relevant to a variety of human malignancies, as well as to have ramifications in cancer pain. Therefore, pharmacological interventions, against NT signaling this time, have the potential not only to target cancer cells directly, but also to inhibit neurogenesis and its stimulatory impact on cancer progression and pain.

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Neurotrofyny: ewolucja podejść do eksperymentalnej naprawy ośrodowego układu nerwowego

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Słowa kluczowe: NGF, BDNF, receptory Trk, LNTR-p75, wektory AAV, uszkodzenie mózgu, przeciwenie rdzenia kręgowego, motoneuron

Podziękowania: Wyrażam wdzięczność profesor Barbarze Oderfeld-Nowak za jej intuicję naukową i wprowadzenie mnie w dziedzinę badań nad czynnikami troficznymi oraz profesor Julii Czarkowskiej-Bauch za 20 lat wspólnych, owocnych badań nad neurotrophiczną kontrolą funkcji i procesów naprawczych rdzenia kręgowego. Mój wkład w dziedzinę i treść tego artykułu nie byłyby możliwe, gdyby nie badania przeprowadzone wspólnie z kolegami z Pracowni Neurochemii i Pracowni Procesów Reinnerwacyjnych w Instytucie Nenckiego, z naszymi doktorantami i doskonałymi technikami. Dziękuję Benjunowi Ji za pomoc w opracowaniu spisu literatury. Praca powstała przy wsparciu finansowym z funduszu na działalność statutową Instytutu Nenckiego.

STRESZCZENIE

Historię badań nad rolą neurotrofin (NT) w układzie nerwowym zapoczątkowało odkrycie przez Ritę Levi-Montalcini w 1952 roku i scharakteryzowanie przez Stanleya Cohena czynnika wzrostu nerwów (NGF), wyizolowanego z tkanki mysiego mięsaka. Obserwacje, pochodzące z badań nad czynnikami troficznymi oraz profesor Julii Czarkowskiej-Bauch za 20 lat wspólnych, owocnych badań nad neurotrophiczną kontrolą funkcji i procesów naprawczych rdzenia kręgowego. Właściwa na tej podstawie wykazują obecność NT i innych cząsteczek, np. metaloproteaz, kontrolujących wzrost włókien nerwowych i przebudowę macierzy pozakomórkowej w tkankach guzów, były dla wielu badaczy bodźcem do zbadania roli tych cząsteczek w fiziologii i patologii układu nerwowego. Czynniki troficzną są od dawna identyfikowane jako białka regulujące neurogenezę i regenerację podczas rozwoju i po uszkodzeniu układu nerwowego. Tysiące badań przeprowadzonych od wcześniejszych lat sześćdziesiątych dostarczyły mocnych dowodów na to, że ta mała rodzina białek jest niezbędna do utrzymania komórek ośrodkowego i obwodowego układu nerwowego oraz do utrzymania długotrwałego dźwięku komórek nerwowych i dźwięku komórek. Często jest używana zarówno w celach regeneracyjnych, jak i klinicznych. Problem, który towarzyszy większości ekskerymentalnych terapii z NT jest to możliwość aktywacji NT w tkankach guzów, a także możliwości w utrzymywaniu komórek ośrodkowego i obwodowego układu nerwowego. Często jest używana zarówno w celach regeneracyjnych, jak i klinicznych. Problem, który towarzyszy większości ekskerymentalnych terapii z NT jest to możliwość aktywacji NT w tkankach guzów, a także możliwości w utrzymywaniu komórek ośrodkowego i obwodowego układu nerwowego. Często jest używana zarówno w celach regeneracyjnych, jak i klinicznych. Problem, który towarzyszy większości ekskerymentalnych terapii z NT jest to możliwość aktywacji NT w tkankach guzów, a także możliwości w utrzymywaniu komórek ośrodkowego i obwodowego układu nerwowego. Często jest używana zarówno w celach regeneracyjnych, jak i klinicznych. Problem, który towarzyszy większości ekskerymentalnych terapii z NT jest to możliwość aktywacji NT w tkankach guzów, a także możliwości w utrzymywaniu komórek ośrodkowego i obwodowego układu nerwowego. Często jest używana zarówno w celach regeneracyjnych, jak i klinicznych. Problem, który towarzyszy większości ekskerymentalnych terapii z NT jest to możliwość aktywacji NT w tkankach guzów, a także możliwości w utrzymywaniu komórek ośrodkowego i obwodowego układu nerwowego. Często jest używana zarówno w celach regeneracyjnych, jak i klinicznych. Problem, który towarzyszy większości ekskerymentalnych terapii z NT jest to możliwość aktywacji NT w tkankach guzów, a także możliwości w utrzymywaniu komórek ośrodkowego i obwodowego układu nerwowego. Często jest używana zarówno w celach regeneracyjnych, jak i klinicznych. Problem, który towarzyszy większości ekskerymentalnych terapii z NT jest to możliwość aktywacji NT w tkankach guzów, a także możliwości w utrzymywaniu komórek ośrodkowego i obwodowego układu nerwowego. Często jest używana zarówno w celach regeneracyjnych, jak i klinicznych. Problem, który towarzyszy większości ekskerymentalnych terapii z NT jest to możliwość aktywacji NT w tkankach guzów, a także możliwości w utrzymywaniu komórek ośrodkowego i obwodowego układu nerwowego. Często jest używana zarówno w celach regeneracyjnych, jak i klinicznych. Problem, który towarzyszy większości ekskerymentalnych terapii z NT jest to możliwość aktywacji NT w tkankach guzów, a także możliwości w utrzymywaniu komórek ośrodkowego i obwodowego układu nerwowego. Często jest używana zarówno w celach regeneracyjnych, jak i klinicznych. Problem, który towarzyszy większości ekskerymentalnych terapii z NT jest to możliwość aktywacji NT w tkankach guzów, a także możliwości w utrzymywaniu komórk