Review Article • DOI: 10.2478/s13380-012-0047-4 • Translational Neuroscience • 3(4) • 2012 • 399-412

Translational Neuroscience

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NEUROGENIC NEUROPROTECTION: FUTURE PERSPECTIVES

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Abstract

Introduction: Neurogenic neuroprotection elicited by deep brain stimulation is emerging as a promising approach for treating patients with ischemic brain lesions. In rats, stimulation of the fastigial nucleus, but not dentate nucleus, has been shown to reduce the volume of focal infarction. Protection of neural tissue is a rapid intervention that has a relatively long-lasting effect, rendering fastigial nucleus stimulation (FNS) a potentially valuable method for clinical application.

Methods: We review some of the main findings of animal experimental research from a clinical perspective.

Results: Although the complete mechanisms of neuroprotection induced by FNS remain unclear, important data has been presented in the last two decades. The acute effect of electrical stimulation of the fastigial nucleus is likely mediated by a prolonged opening of potassium channels, and the sustained effect appears to be linked to inhibition of the apoptotic cascade.

Conclusion: A better understanding of the cellular and molecular mechanisms underlying neurogenic neuroprotection by stimulation of deep brain nuclei, with special attention to the fastigial nucleus, can contribute toward improving neurological outcomes in ischemic brain insults.

Keywords

• Neurogenic neuroprotection • Fastigial nucleus • Stroke • Cerebrovascular accident • Subarachnoid hemorrhage

Received 12 September 2012
accepted 05 November 2012

Introduction

The protection of brain tissue after traumatic or ischemic events poses a current challenge for critical neurological care in medical centers worldwide [1-5]. The goal of neuroprotection is to preserve viable areas of brain tissue in the event of injury in order to restore performance to levels as close as possible to their original physiological functions. This approach constitutes a way of reestablishing the micro-environment and conditions needed for suffering brain neurons to inhibit intrinsic undesirable programmed processes of cell death. Reduction of cellular metabolism and stimulation of trophic properties in these cells is conducive to achieving a new balance for cellular viability. To this end, many types of pharmacological and physical interventions have been employed in intensive care units [1-9]. However, real benefits of the current treatments are far from ideal, particularly given the difficulties in inhibiting the inexorable cascade triggered by the initial event and in controlling the complications associated with treatment options [4].

Neurogenic neuroprotection opens up a new frontier for the preservation of the penumbra area in ischemic brain of humans. For the purposes of this review, the penumbra area has been defined as the risk zone for cellular death that is sensitive to therapeutic procedures (Figure 1) [10-17]. There are no anatomical markers that clearly map the penumbra region, and its borders may overlap with the adjacent necrosis area. This morphological feature indicates the dynamic cellular and molecular events occurring in the penumbra area that can drive some of its regions toward the necrotic center or render them resistant to secondary neurodegeneration. Nevertheless, a detrimental scenario can be modified by introducing procedures that are able to change the apoptotic cycle of damaged cells.

Robust data shows that electrical stimulation of cerebellar fastigial nucleus (FN) can elicit marked global protection against brain injury in rats submitted to different types of hypoxic, ischemic and excitotoxic injury [18-22]. Long-lasting neuroprotective effects have been evidenced that persisted for up to 10 days after nuclei fastifial nucleus stimulation (FNS) [23]. Moreover, a 50% reduction in neuronal death has been reported following induced ischemic injury, 3 days after stimulation of FN deep in cerebellum [18], thus revealing a promising method for controlling secondary neuron degeneration in brain ischemia.

Experimental evidence

The cerebellar nuclei were first recognized by Raymond de Vieussens [24] and called the emboliform, globose and fastigial nuclei by Stilling in 1864 [25]. However, evidence of changes in the control of systemic and encephalic blood flow after stimulation or lesion of cerebellum nuclei dates back to 1969 [26-28]. These observations led to the notion of a possible effect of FNS on adjacent areas of brain infarct [22]. FNS for 1 hour in rats has been shown to reduce infarction volume by 50% compared to non-stimulated control animals [18,20-22,29]. The region of spared tissue is referred to as the area of penumbra, whose neurons enjoy neuroprotection through

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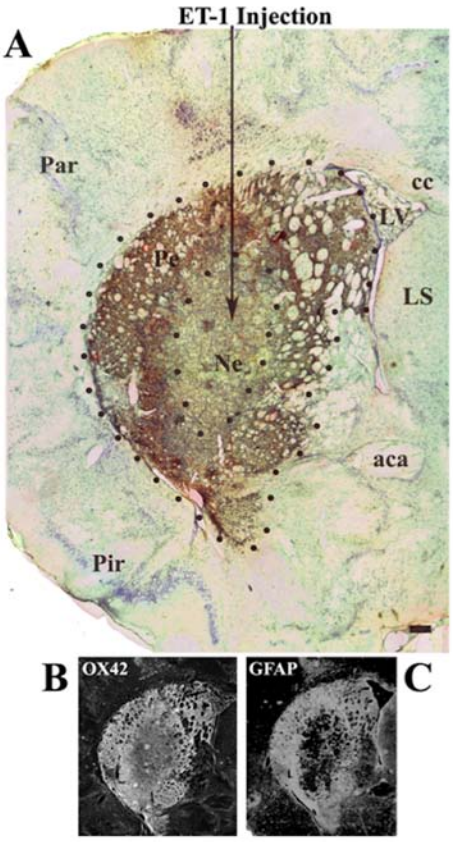

Figure 1. Figure shows superimposed images of two coronal, adjacent brain sections of rats 15 days after a striatal injection of endothelin-1 (ET-1). ET-1, a potent vasoconstrictor, is employed to promote focal and permanent experimental ischemia in brain parenchyma with formation of a necrosis zone and a surrounding penumbra region. One section was stained for OX-42 immunoreactivity (brownish color, a marker for activated microglia/macrophages) and counterstained with cresyl violet (bluish color) for visualization of *inter alia* neuronal/glial cell nuclei. Adjacent section was stained for glial fibrillary acidic protein (GFAP) immunoreactivity (reddish color, a marker for activated astrocytes). The image layer of astroglial staining with 52% transparency was placed over the microglial macrophage/cresyl violet staining. The internal dots surround the necrosis area (Ne), while the external dots surround the penumbra area (Pe). The panels B and C are the dark field images of the OX-42 and GFAP immunolabelings shown in panel A. Note the presence of small infiltrating cells stained for cresyl violet in the necrosis region, also possessing OX-42 immunolabeling, thus being of macrophage nature (A, B). Few GFAP positive astrocytes are seen in the necrosis region (A, C). In the penumbra area, massive infiltrations of microglia/macrophages and astrocytes coexist with surviving neurons (not shown). The molecular events that take place in the penumbra area trigger neuronal/non neuronal cell death or salvage, leading to the region being incorporated into the necrosis area or undergoing the process of neuroplasticity-induced neurofunctional restoration, respectively. Abbreviations: ET-1, endothelin-1; Par, parietal cortex; cc, corpus callosum; aca, anterior commissure; Pir, piriform cortex; Ne, necrosis area; Pe, penumbra area. Scale bar = 200μm.

inhibition of the secondary neurodegeneration process [19]. Although the effects of experimental FNS have been well established, the mechanisms underlying this neurogenic neuroprotection remain a topic of intense investigation [30].

Neuronal circuitry

The electrical stimulation of FN influences local neurons as well as the abundant fibers of passage within the nucleus and surrounding area [31]. Stimulation of the rostromedial portion of the cerebellar FN in rat triggers a stimulus-locked elevation of arterial pressure and heart rate [32-35]. This response, discovered in 1969 by Miura and Reis [28] and Achari and Downman [26] in anesthetized cats, is known as "the fastigial pressor response" (FPR). In contrast, microinjection of excitatory amino acids, which only excite neuronal perikarya, decreases arterial pressure and heart rate [33,36,37]. These observations can explain the apparent paradoxical findings seen after indiscriminate FN electrical stimulation. Nevertheless, the excitation of passage fibers seems to be as essential as the stimulation of intrinsic neurons in evoking neurogenic neuroprotection [18]. Since the effects in the brain appear to be global, the network responsible for this action might consist of widespread brain projections from the nucleus [31]. However, this is not the case for FN, which possesses relatively short projections into the vestibular nucleus, reticular dorsal and paramedian formation, many pontine nuclei including the locus coeruleus, the parabranchial nucleus as well as the centromedial thalamic nucleus and parafascicular complex, substantia nigra and amygdala [28,30,38-41].

The same neuroprotective response observed after stimulation of the FN was

also evoked by stimulation of the dorsal periaqueductal gray matter (DPAG) area and subthalamic vasodilator area (SVA) [42,43]. One possibility is that DPAG stimulation-induced conditioned neuroprotection is mediated by intrinsic FN neurons (Figure 2). Some studies have revealed that FN efferents, labeled with radioactive amino acids, project into the lateral edge of the PAG in monkeys and dogs [39,44], while other authors have shown that caudal FN regions involved in oculomotor systems also have fine fibers that extend into the PAG [45]. Additionally, the PAG also has extensive ascending projections to the midline, intralaminar, and reticular thalamic nuclei, as well as the hypothalamus and basal forebrain [46-49]. The presence of these projections supports the notion that the DPAG excites the neuroprotective response of SVA, which is independent from the FN [42].

The existence of a trans-synaptic neuronal network, currently acknowledged from a functional point of view, is consistent with the widespread neuroprotective effect of FN stimulation [30]. As the brain is highly dependent upon a continuous and abundant supply of oxygen and glucose for its metabolism, even modest reductions in regional cerebral blood flow (CBF) impair neuronal function and, if briefly sustained, result in neuronal death. Notwithstanding, there are naturalistic behaviors in which regional CBF may fall below thresholds that would normally compromise neuronal function. The fact that these reductions of regional CBF are associated with stereotyped patterns of behavior, as observed in hibernating mammals or diving vertebrates, implies the presence of neuronal networks dedicated to self-protection [50]. Therefore, the findings regarding the FNS were attributed by some authors [20] to phylogenetic maintenance of this self-protection circuitry. On the other hand, the stimulation pattern used in the cited studies is not normally observed physiologically [18-21]. Perhaps these experimental studies are describing the effect of neuronal circuit hyperactivation and its physiological basis, pointing to the principles of the coupling between neuronal activation and increased regional cerebral blood flow.

In this sense, functional magnetic resonance imaging (fMRI) has advanced to become one of the leading tools for assessing brain function [51,52]. Because fMRI is based on the blood oxygenation level dependent (BOLD) effect, it does not directly record neural activity. Although generally accepted in the literature, there is no hard scientific evidence that the BOLD effect is exclusively a consequence of the regional increase in neuronal metabolism. The forgotten hypothesis, which holds that vasodilation and the consequent BOLD contrast can be triggered by the brain network itself, is totally plausible. Moreover, distinguishing BOLD signals created by cortical projection neurons from those produced by intracortical neurons has proved difficult [51,52]. The existence of neuronal circuitry that controls brain microcirculation, linked to its function,

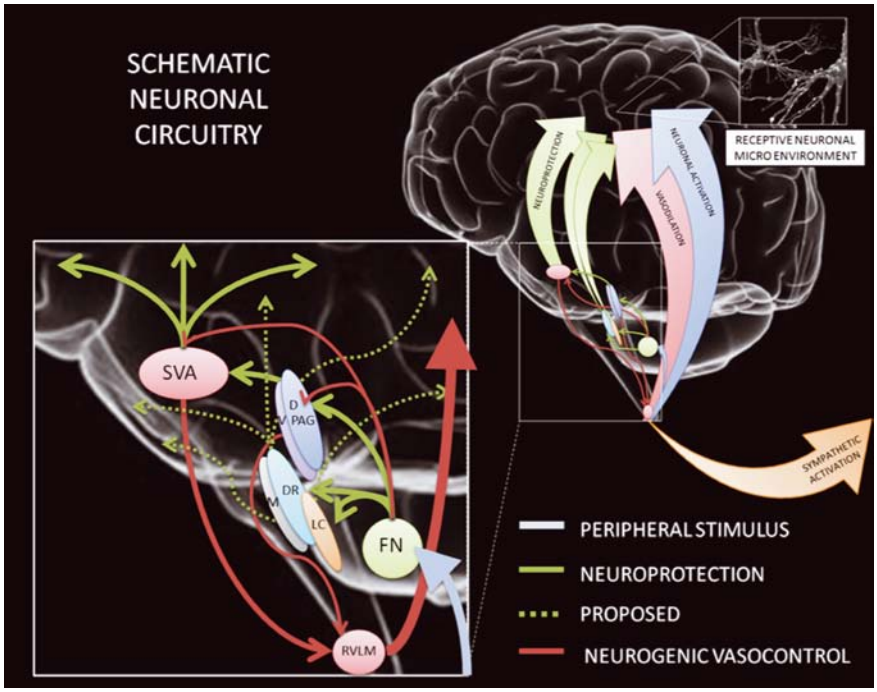

Figure 2. The neuroprotection circuitry might encompass neuronal circuitry involved in the coupling between neuronal activation and its consequent energetic demands. (1) Excitation of neurons and/or fibers projecting through the subthalamic vasodilator area (SVA) reduces ischemic infarctions to the same degree as excitation of the fastigial nucleus (FN) neurons. (2) Conditioned neuroprotection is independent of increased cerebral blood flow (CBF). The effects are long-lasting and not attributable to changes in blood gases, brain temperature, or rat strain. (3) The neuroprotective effects of SVA and FN stimulation are mutually independent, and FN-evoked cerebrovasodilation is mediated by SVA neurons. (4) Both the systemic and cerebrovascular components of FN stimulation are abolished by bilateral lesions of the rostral ventrolateral medulla (RVLM) [64,65]. (5) The SVA also mediates the primary elevations of CBF elicited by hypoxic excitation of the sympathoexcitatory neurons of the RVLM. (6) Intrinsic neurons of D- and VPAG differentially regulate CBF. (7) Neurons of DPAG are mediating neuroprotective effects, independently of changes in CBF and/or arterial pressure.

is not a new concept and there is sound experimental data supporting this theory.

Two lines of evidence indicate that specific nuclei are essential for the expression of cerebrovascular vasodilation. First, electrical stimulation of the rostral ventrolateral medulla (RVLM) in intact or spinalized rats, site-specifically and dose-dependently, elevates regional CBF but not regional cerebral glucose utilization (CGU) [53], thereby replicating hypoxic vasodilation [54]. This response can only be attributed to stimulation of the reticulospinal sympathoexcitatory neurons since these are the only neurons in the region excited by the hypoxia [55]. Second, bilateral lesions of RVLM yet not of adjacent regions, reduce the elevation of regional CBF caused by hypoxemia by over 50%. The fact that such lesions do not affect the vasodilation elicited by hypercarbia indicates that the response is stimulus elective [53,56]. Therefore, cerebrovascular vasodilation elicited in the cerebral cortex by hypoxemia is largely a response to excitation of oxygen-sensitive brainstem neurons [55-58], as opposed to a direct effect of hypoxia on blood vessels or stimulation of arterial chemoreceptors whose activity, while regulating blood flow to most vascular beds has no effect on cerebral circulation.

In this context, the circuitry involved in neurogenic neuroprotection could represent the missing link. Descending neuronal projections from the DPAG innervate the parabrachial, ventromedial, and ventrolateral medulla with few direct projections into the spinal cord [59-63]. Earlier observations suggest that stimulation of the RVLM might produce some neuroprotective effects (~25%) [29,53]. It is possible that through these direct projections, DPAG might excite the RVLM [61,62]. On the other hand, the effect observed in the more recent experiments is much more prominent (~50%) and independent of sympathoexcitation, which occurs in response to RVLM stimulation [60,64]. By comparison, the non-significant neuroprotection observed in response to VPAG stimulation may be explained by the excitation of DPAG projections to the RVLM.

Additionally, both the systemic and cerebrovascular components of FNS are abolished by bilateral lesions of the RVLM [64,65]. The neuronal elements within the FN responsible for mediating the FPR appear to be axons of brainstem neurons that project via collaterals to innervate both cerebellum and RVLM [66]. Similarly, the SVA also mediates the primary elevations of CBF elicited by hypoxic excitation of the sympathoexcitatory neurons of the RVLM without compromising the cerebral vasodilation evoked by hypercarbia [67,68]. Taken together, this evidence indicates that the neuroprotective circuitry produces collaterals in order to promote changes in CBF. The functional significance of this coupling might represent the neuronal circuitry involved in the control of regional blood flow and its relationship to regional neuronal activation and synchronization in a given behavior [31,60,69]. Alternatively, there is no evidence of a topographical distribution of the diffuse projections described earlier in the text. This is a critical step toward explaining locoregional increase of blood flow in the brain.

Neurogenic neuroprotection

The cognizance of this entity was demonstrated after extensive studies involving stimulation of FN and its consequent modification of CBF dynamics [26,27,33,35,70,71]. Previous reports have demonstrated the ability of FNS to induce changes in arterial pressure at rest and in reflex control [26-28,70]. Furthermore, there is a global rise of CBF during the FNS, an event that is not accompanied by an increase in the CGU, thus suggesting an absence of functional activation [29,35]. The first hypothesis was the improvement of blood circulation through collateral arteries in the ischemic penumbra, without associated increase in local functional demand, leading to beneficial action on the vascular ischemic lesion [22]. Although CBF is higher in non-ischemic areas bilaterally, the FNS does not raise CBF in the penumbra area of ischemic infarctions [29]. Consequently, a different mechanism to that linked to CBF variation may be responsible for the neuroprotection phenomenon.

In order to establish the physiological role of neurogenic neuroprotection, some authors have sought to link CBF to cerebral metabolism [29,35]. The penumbra area presents lower CBF with regard to its cellular metabolism [10,11,16,72], creating a "misery perfusion" as described by Baron et al. [72] in 1981. Conceptually, reduced cellular metabolism in damaged areas could restore the balance needed for cellular viability and explain the mechanism of neurogenic neuroprotection. Notwithstanding, the FNS fails to reduce glucose utilization in penumbra areas, yet raises it in non-ischemic zones [31,35,73].

The current evidence indicates that neuroprotection triggered by FNS is independent from CBF changes or cellular energetic consumption at the ischemic penumbra [29]. Moreover, several experimental studies have failed to demonstrate the relationship between neuroprotection and blood pressure variations, hematocrit levels, body temperature and blood gas concentration [43]. However, electrical stimulation of FN does create a specific sympathetic-excitatory effect along with increased arterial blood pressure and cerebral blood flow, tachycardia and activation of predatory behaviors [26,70]. These neurovegetative effects are not associated with a rise in metabolism [27,28,35,43,71,74]. In contrast, the exclusive stimulation of intrinsic neurons of FN evokes a sympathetic-inhibitory effect characterized by hypotension, bradycardia and global reduction of CBF [33,43,73]. Moreover, the selective injury of these neurons makes FNS non-neuroprotective, thus suggesting the association of sympathetic inhibition and the neuroprotection effect.

Drawing on the previous knowledge that sympathetic inhibition is triggered by the stimulation of the ventral portion of periaqueductal gray matter (VPAG), Glickstein et al. [43] evaluated the stimulation of this region in the context of post-ischemic neuroprotection. If the association between sympathetic inhibition and neuroprotective effect were to prove valid then at least in theory, brain neurovegetative effects would be causative regardless of their source. However, although Glickstein and collaborators achieved inhibition of the encephalic sympathetic system, chemical stimulation of intrinsic neurons of the ventral portion of PAG failed to produce neuroprotection [43]. These observations

suggest that sympathetic inhibition is not a pre-requisite for the neuroprotection induced by electrical stimulation of deep nuclei. Another important finding of this study was that the neuroprotective effect from FNS resembled that produced by the same procedure applied to the DPAG.

The conditioned neuroprotection produced by stimulation of the DPAG bears similar features to the conditioned neuroprotection evoked by FNS and SVA [42]. First, the infarction volume is decreased by ~50% even when occlusion is performed 72 h after the stimulation. Second, conditioned neuroprotection is independent of increased CBF. Third, salvage can be initiated by using similar parameters for electrical stimulation of the DPAG, FN, and SVA [31,42]. This similarity suggests the involvement of related intrinsic brain systems to evoke conditioned neuroprotection.

Other evidence also points to a positive effect of central electrical stimulation at the tissue level. The stimulation of the SVA significantly reduced the volume of focal infarction (by 58%) of brain ischemic injury in rat after three days [42]. Nevertheless, no change in neuroprotective effect was detected in FNS-animals that had damaged SVA. Likewise, SVA stimulation in animals with damaged FN resulted in no changes in infarction volume within the ischemic penumbra. Moreover, the most important observation was the counteraction of the FNS-induced increase of cerebral blood flow in the SVA-injured rats. Taken together, these observations have experimentally confirmed that neuroprotection evoked by FNS is not dependent on changes in CBF. This data suggests the existence of an intrinsic neuroprotective network with mechanisms of actions that involve neurons themselves.

This hypothesis was corroborated by Golanovet and collaborators [69], who demonstrated spontaneous fluctuations of regional CBF in anesthetized rats. These fluctuating waves occur at a reasonably constant frequency of 6 events per minute with substantial increase in CBF compared to basal values. In addition, characteristic changes in electrographic activity precede the elevation of regional CBF [69]. The peak of electrographic activity precedes (by approximately 1.5 seconds) the transient elevation of CBF (Figure 3). Electrical stimulation of the FN evokes exactly the same result. The hyperactivation of this circuitry may explain the neuroprotective effect seen by altering the electrophysiological activity of the cerebral cortex, and consequently by inducing cellular changes.

The electrical instability generated by focal ischemia has previously been described in the correspondent ischemic penumbra in the form of the rapid appearance of repeated depolarizing waves, also known as peri-infarction depolarizing waves (PID) [75-78]. PID broaden throughout the cortex in the same manner as cortical spreading depression outside the infarct region. This phenomenon is initiated by acute depolarization and then maintained by secondary waves of similar electrophysiological properties, which travel slowly in normal cortex [75-78]. PID are now believed to contribute to tissue damage by increasing the metabolic demand of already compromised local cells, limiting the energy available for cellular membrane repolarization.

At both electrophysiological and pharmacological levels, the neuroprotective effect of FNS is reversible by a previous intraventricular injection of potassium channel blocker (e.g. glibenclamide), when the stimulus is prompted immediately before the middle cerebral artery occlusion in rats [79]. This finding is consistent with the possibility that FNS can evoke a prolonged opening of potassium channels acutely, leading to hyperpolarization and reduced neuronal excitability. In addition, glibenclamide may enhance cortical spreading depression-associated hyperemia [80] and Golanovet and collaborators [76] corroborated this theory demonstrating that the FNS can reduce PID by prolonging the latency of its appearance and reducing the frequency of events. However, glibenclamide has failed to revert neuroprotection when administered immediately after the middle cerebral artery occlusion in rats, submitted to stimulation three days earlier. This data suggests the existence of at least two possible cellular mechanisms involved in neurogenic neuroprotection (Figure 4). While a possible acute mechanism may involve the modulation of potassium channels, thus accounting for rapid but short-lived neuroprotection, a subsequent and long-term response may depend on intracellular signaling and changes in gene expression. In addition, FNS-mediated responses may modify the apoptotic cascade flow. Furthermore, acute excitability suppression may not constitute a critical event underlying neuroprotection, as demonstrated in stimulated rats that showed inhibition of spreading depolarization waves [76]. The changes in excitability could be an epiphenomenon or alternatively, be part of the neuroprotection initiation process.

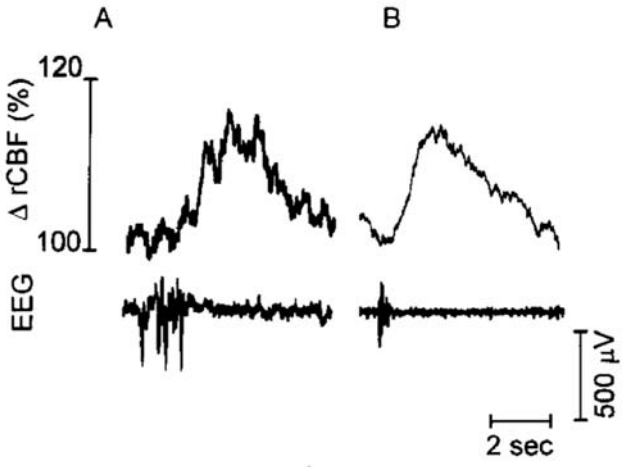

Figure 3. Characteristic burst-wave complexes recorded in parietal cortex of anesthetized rat. Upper row: (A) individual and (B) averaged (n = 25 sweeps) bursts recorded at slow sweep-speeds followed by single wave of vasodilation. Note that after averaging, the afterpotential of individual bursts disappears, while only the initial potential remains. With permission from ref. [31].

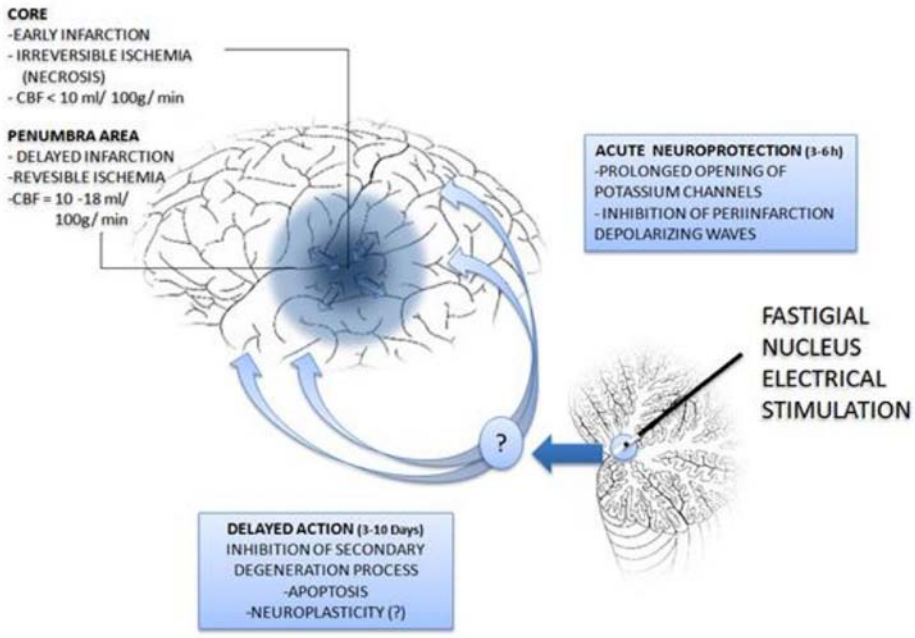

Figure 4. A 1-h electrical stimulation of fastigial nucleus (FN) (1 s on, 1 s off; 0.5 ms pulse durations (75–150 mA)) reduced infarctions triggered by permanent occlusion of the middle cerebral artery by 48–55% in Sprague–Dawley rats and by 59% in Fisher rats [42]. The salvaging effect of FN stimulation was long-lasting and reduced the volume of infarctions 72 h or 10 days later by 58 and 26%, respectively, in Fisher rats [42]. As the effect appears to be global in the brain and given that FN possesses relatively short projections, other relays must be involved in this network.

Intrinsic regulation of brain inflammatory responses

It has long been recognized that the threshold for inflammation to occur in brain is higher than in other tissues [81]. Some authors however, have suggested that perturbation or dysfunction of certain brain areas could be a contributory factor to the initiation, progression, or lack of resolution of inflammatory responses [82-85]. Ischemia triggers an inflammatory process in which capillaries, the blood-brain barrier, play a specific role. Also, ischemia induces the production of several pro-inflammatory cytokines, such as IL-β [12,85,86], which may be involved in the expression of isoform-2 of nitric oxide synthase (iNOS or NOS2) [87-89] and of the adhesion molecule ICAM-1 [90,91] in local capillaries. The blockade of expression of these components seems to be able to reduce the volume of ischemic infarction [34,91,92]. Therefore, these mechanisms have been explored in order to understand the effect of FNS at a molecular level.

Electrical stimulation of the FN prior to the ischemic episode reduced *NOS2* mRNA and protein expression by over 90%, where this decrease was restricted to the penumbral area [84]. These results demonstrated that FNS is able to modify ischemia-dependent inflammatory gene expression, suggesting that the neuroprotective effects of FNS could be due, in part, to attenuation of *NOS2* expression and of other pro-inflammatory molecules [84]. However, whether decreased *NOS2* expression was a direct consequence of FN activation (i.e. FNS reduced the capacity of the microvasculature to express *NOS2* or the presence of *NOS2*-expressing leukocytes) or was secondary to the ability of FNS to reduce ischemic damage was not established.

In order to distinguish between these two mechanisms, the effects of FNS on cerebrovascular inflammatory responses were measured in the absence of ischemia. Galea et al. [85] demonstrated that brain microvasculature of rats submitted to FNS becomes refractory to inflammation generated by the presence of IL-β, by reducing the ability of cerebral microvessels to express both cell adhesion molecules as well as pro-inflammatory molecules, including NOS2. Furthermore, increased nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha (IκBα) mRNA induction suggests that FNS modifies the transcriptional machinery involved in IκBα expression (also known as an inhibitory protein that prevents activation of nuclear factor kappa-light-chain-enhancer of activated B cells, NF-κB, which controls the transcription of DNA in cellular responses to various stimuli).

Apoptosis

The penumbra area has a partially preserved blood supply and is hypermetabolic, which evokes sublethal damage ultimately promoting a delayed apoptotic cell death [93-95]. Apoptosis is a cell-intrinsic process that is essential for animal development and tissue homeostasis [96]. As the tissue salvaged by FNS after focal ischemic insult follows the contours of the presumed ischemic penumbra, the hypothesis that FN not only suppresses inflammatory reactions [84,85] but also attenuates apoptotic processes gains merit.

One evolutionarily conserved component in the apoptotic pathway is the involvement

of the members of the caspase family [97]. The measurement of caspase activation serves as a valid early apoptotic marker, while morphological change occurs later as a result of caspase activation [98]. Caspase-3 activity is low in freshly isolated brain slice cultures, and slowly increases in a linear fashion over a 24-h period most likely through spontaneously occurring apoptosis [99]. In contrast, robust caspase-3 activation is seen when the slice cultures are challenged with an apoptosis inducer such as staurosporine. This increases intracellular calcium, reactive oxygen species [100,101] and the release of mitochondrial cytochrome c with subsequent activation of caspase-9 and caspase-3 [102-104].

The activation of caspase-3 was suppressed in brain tissues removed 72 h post-FNS compared with dentate nucleus (DN)-stimulated controls [99], indicating that FN stimulation suppresses apoptosis. Nevertheless, the author found no reproducible or consistent pattern of changes in levels of Bcl-2 family proteins between samples from both FN- and control DN-stimulated animals [99]. Therefore, changes in the overall levels of Bcl-2 protein family members are unlikely to contribute to the enhanced mitochondrial resistance conferred by FN stimulation [105]. On the other hand, FN stimulation decreases Bax insertion into mitochondria [105], a critical step in the initiation of apoptosis [106,107]. Although observations in isolated mitochondria may not be directly applicable to the experiments in which Bax insertion was investigated in brain slices, these findings may provide a molecular link between FNS and reduction in caspase-3 activation [99,105].

FNS also reduces staurosporine-induced cytochrome c release from mitochondria compared with DN-stimulated rats [99]. This may result from a direct effect of FNS, rendering the mitochondria more resilient to the insult. Alternatively, there may be an indirect effect of FNS of increasing the threshold of cellular stress essential to evoke mitochondrial dysfunction. Seeking insights into the molecular mechanisms of FNS, Zhou and collaborators used isolated rat brain mitochondria to demonstrate that FNS increases the ability of mitochondria to maintain their functional state during Ca^{2+} loading, and inhibits mitochondrial pathways from triggering apoptosis [105].

Mitochondria have emerged as critical organelles in determining cell fate [108]. During ischemia and excitotoxic injury, Ca^{2+} enters neurons through NMDA receptors [109,110], and the overloading of Ca^{2+} into mitochondria leads to the opening of the permeability transition pore and loss of mitochondrial membrane potential [111,112]. As a result, the outer mitochondrial membrane (OMM) becomes permeable to intermembrane space proteins, such as cytochrome c, which marks the beginning of mitochondrial dysfunction [113]. Interventions enhancing OMM integrity would clearly offer neuroprotection against injury.

Zhou et al. [105] used mastoparan and Ca^{2+} as inducers of cytochrome c release. Earlier studies by others had shown that mastoparan induces the mitochondrial permeability transition [114] and releases mitochondrial proteins [115]. FN-conditioned mitochondria release less cytochrome c in response to mastoparan and Ca^{2+}, suggesting that FNS is able to stabilize permeability transition pores (PTP) and enhance the integrity of the OMM. This ability attenuates the inhibition of respiration produced by Ca^{2+} loading, maintains adenosine-5′-triphosphate (ATP) production, and may reduce free radical production during ischemia or excitotoxicity. Taken together, these data provide evidence that the neuroprotection exerted by FNS is mediated by an effect on mitochondria, resulting in enhanced Ca^{2+} buffering capability, improved respiration in the presence of excess Ca^{2+}, reduced Bax insertion, and attenuation of cytochrome c release [105].

This suppression of apoptosis by FNS was moderate, leading to reductions of approximately 30% compared with DN-stimulated controls, a considerably lower level than anticipated from *in vivo* studies, where maximum reductions in cell death are greater than 50% [18,21,23]. This may reflect the nature of the *in vitro* system, in that adult neurons are fragile in culture, so the full protective potential of FNS is not completely displayed in this system. As FN neuroprotection is at least in part attributed to the prevention of caspase-activation primarily in glial cells [99], changes in the micro environment may also play an important role. On the other hand, *in vivo* neuroprotection by FNS may involve multiple protective mechanisms besides anti-apoptosis, such as the systemic suppression of inflammatory responses [84,85].

Perspectives

The experimental design of the reports cited above adopted the stimulation-followed-by-lesion paradigm, which seems to be of little value from a clinical point of view. To identify endogenous mechanisms of protection and repair, and to make use of these mechanisms therapeutically, biomedical investigators have developed preconditioning strategies. Classically, preconditioning denotes a procedure through which a noxious stimulus, close to but below the threshold of damage, is applied to the tissue. However, there are other ways of "preconditioning" the brain. Shortly after preconditioning or after a delay, the organ (and therefore the organism) develops resistance or tolerance to the same, similar, or even different noxious stimuli given beyond the threshold of damage. Preconditioning thereby protects against subsequent injury. In this context, FNS can be considered a preconditioning measure that does not run the risk of sublethal damage. On the other hand, FNS can also be used to change the chain of events when still reversible.

Ischemic events due to vasospasm

Alterations in CBF and metabolism after subarachnoid hemorrhage (SAH) are well known and have been extensively described [3]. Cerebral vasospasm remains one of the most serious complications after SAH [7,116,117]. This is the classic cause of delayed neurological deterioration after aneurysmal subarachnoid hemorrhage that leads to cerebral ischemia and infarction with poor outcomes, and occasionally to death [117]. Cerebral vasospasm consistently fails to respond to treatment, emphasizing the need for further research into the underlying mechanisms of SAH-induced cerebrovascular dysfunction. Over the past decades, several

pharmacological approaches have been investigated for the prevention of cerebral vasospasm [3,118,119]. Despite advances in the understanding of the pathophysiological nuances of cerebral vasospasm, it has been correlated with a 1.5- to threefold increase in death within the first 2 weeks of the ictal event [119]. Unfortunately, no efficacious treatment is available for this critical patient group [6-8,118].

Classically, vasospasm follows a typical time course, in that its onset usually occurs within 1 week of the hemorrhage, reaches maximum severity between days 7 and 14 post-SAH, and usually dissipates after 14 to 28 days [3,117-120]. The most consistent predictor of vasospasm has been the extent of SAH seen on post-ictal computed tomography (CT) scan [121-123]. However, a statistically significant association between the extent of subarachnoid blood and subsequent development of vasospasm was observed only if the initial CT imaging study was performed within 24 h of aneurysmal rupture [124]. Radiographically, apparent vasospasm develops in approximately 60 to 70% of all patients with aneurysmal SAH. Of these cases, two thirds suffer ischemia sufficiently severe to cause transient or permanent neurological deficits, so-called symptomatic or clinical vasospasm. Although the ability to predict vasospasm is a powerful tool for the neurointensivist, neurosurgeons' hands remain tied.

Brain preconditioning in order to treat the consequences of vasospasm is not a new idea. Electrical stimulation of the spinal cord (SCS) with epidural electrodes has been used to treat chronic pain syndrome and peripheral vascular disease [125-127]. In addition to its effects on the peripheral vasculature, cervical SCS seems to have a similar effect on the cerebral vasculature. Since the first report by Hosobuchi in 1985 [128], the ability of SCS to augment CBF has been demonstrated in several clinical and experimental studies. [129-135]. A number of clinical reports in which authors describe the use of SCS to treat patients with cerebral ischemia have been published [135-137]. However, despite the promise of clinical benefit of SCS in the treatment of cerebral ischemia, the effective use of SCS is hampered by a lack of understanding of its mechanism(s) of action.

Evidence from studies of the peripheral vasculature suggests that SCS produces a reversible functional sympathectomy [32,138-140]. Modulation of the superior cervical ganglion at a cervical-thoracic transitional level, suppressing efferent signals from their origin to the sympathetic chain may be one explanation. Further support for such a pathway stems from the finding that concurrent stimulation of the cervical sympathetic chain overcomes the CBF response to SCS [134]. However, although the sympathetic system may play an important role in the pathogenesis of vasospasms [141], a reversible sympathectomy cannot be considered the sole mechanism involved in mediating both SCS-induced CBF increase and vasospasm prevention effects. The genesis of vasospasm involves vasomotor changes and an inflammatory cascade promoted by the presence of blood in the brain basal cisterns [3,120,123].

Results of a study by Patel et al. [142] demonstrated that surgical interruption of cervical sympathetic outflow has no effect on SCS-induced CBF augmentation. At baseline, surgical sympathectomy did not significantly alter CBF. When SCS was applied after surgical ablation of the superior cervical ganglion, the resultant CBF response remained robust, thus indicating that the CBF response to SCS is not significantly dependent on cervical sympathetic outflow. Furthermore, the effects of SCS on CBF are completely abolished by interruption of the spinal cord pathways above the level of stimulation. SCS performed after spinalization failed to elicit any increase in CBF [142]. These lines of evidence suggest that cervical SCS produces a significant cerebrovascular effect and that this effect may involve alterations in sympathetic tone as well as in indirect activation of brainstem or cerebellar vasomotor centers.

The primary vasodilation that occurs as an oxygen-conserving response, such as in the diving reflex, has been well described in diving vertebrates [50]. It comprises a rapid set of reflex autonomic adjustments in which differentiated excitations of sympathetic neurons is critical. This sympathetically mediated vasomotor response can be replicated by electrical or chemical stimulation of a small population of neurons, mostly adrenergic, lying within a small subnucleus representing the C1 area of the RVLM [143]. These neurons also mediate much of the sympathetic and cerebrovascular response to hypoxemia [144]. These neuronal pathways, through which excitation of oxygen-sensitive neurons in RVLM reflexively protects the cerebral cortex from hypoxia, are indirect [53]. Projection is polysynaptic, reaching cortex through as-yet-undefined projections to upper brainstem and/or thalamus. Additionally, the subcortical-cortical efferent vasodilator pathway does not directly regulate cerebral vessels. Rather, it appears to excite a small population of cortical neurons that seem to be dedicated to transducing a neuronal signal into vasodilation [31,33,53].

This system also appears to relay the central neurogenic vasodilation elicited from other brain regions, including the FN and nucleus tractus solitarii (NTS) areas [145]. Neurons of the RVLM are the principal relay for the potent vasopressor and cerebrovascular vasodilator responses evoked by electrical stimulation of the cerebellar FN [53]. Both the systemic and cerebrovascular components of the fastigial effects are abolished by bilateral lesions of the RVLM [64,65]. The neuronal elements within the FN responsible for mediating the FPR appear to be axons of brainstem neurons that project via collaterals to innervate cerebellum and also RVLM, since microinjection of excitatory amino acids into the FN fails to replicate the FPR [64,65].

However, unlike conditioned central neurogenic protection, activation of neurons of the RVLM appears to be less cytoprotective [29]. Cervical SCS likely has a limited effect only on the RVLM, and does not reflect the true potential of this neuronal circuitry for patients experiencing ischemic suffering due to vasospasm. Deep stimulation of the FN seems to be the best target in order to promote a dual effect on the brain parenchyma: vasodilation and neuroprotection. Experimental investigations on neurogenic neuroprotection demonstrated a maximum effect, regarding the counteraction of neuronal death, provided the electrical stimulation had occurred 3 days before the middle cerebral artery occlusion in rats [20,21]. Knowing that ischemic events due to vasospasm occur at

least 3 days after aneurysm rupture, there may be a therapeutic window, and a new horizon for neurogenic neuroprotection. If experimental results could be extrapolated to clinical situations, then a substantial reduction of neuronal death of about 50% could be achievable in humans, a level not attained by any other pharmacological intervention. Such a fall in mortality would represent a remarkable feat of translational neuroscience leading to a substantial reduction of mortality from brain ischemia in clinical situations.

Malignant cerebral infarctions

Nevertheless, as outlined earlier, there are times when we cannot predict events even in the near future. Malignant middle cerebral artery infarction is a large hemispheric infarction with poor outcome attributable to the ischemic inflammation/edema that causes an early rise in intracranial pressure and subsequent brain herniation and death [1,9,11,12,146]. No clinical therapy has proven effective in preventing neuronal death in the penumbra area thereby improving patient outcome [2,5]. Surgical decompression techniques have been proposed to relieve high intracranial pressure, but this represents only a critical intervention in an inexorable process [146].

The only method currently used to prevent this outcome is the unblocking of the compromised vessel. However, although recombinant tissue-type plasminogen activator (rt-PA) has been approved for acute ischemic stroke, less than 5% of qualifying patients actually receive rt-PA [147,148]. Several factors have been identified to explain the underuse of this therapeutic approach for ischemic stroke: the short therapeutic window, insufficient public warning and knowledge of stroke symptoms, the limited number of centers able to perform thrombolysis on a 24-hour basis, and excessive fear of hemorrhagic complications [149]. Although in clinical practice this complication may be less frequent than failure of treatment to recanalize occluded cerebral artery or early (up to 48 hours) reocclusion, intracerebral hemorrhage seems to represent a significant obstacle to the generalization of thrombolytic therapy [150]. Those patients ineligible for trombolysis are condemned to an ineffective group of systemic measures in the intensive care unit.

Acute intervention by means of FNS, leading to control of the apoptotic cycle of compromised neurons and of the inflammatory cascade taking place in the penumbra area, is feasible in neurosurgical clinical practice and may change the course of this malignant evolution.

Nowadays, the implant of electrodes for stimulation of deep brain nuclei (DBS) is mainly performed in patients with movement disorders and psychiatric disturbances. Despite being held in elective situations, as it has become a common procedure, the application in critical situations should not be an impediment issue. MRI, utilized for anatomical programming, is available in emergency situations, and 3D target localization can be calculated in seconds with computational assistance. Another important point is that although DBS surgery for patients with movement disorders usually implies the need of awaken patients, DBS for neurogenic neuroprotection could be performed safely with patients under general anesthesia. The only variable that must be measured, besides the own anatomy, is the increase of CBF that occurs when we stimulate the FN. This variable can be easily measured by intraoperative transcranial Doppler ultrasound.

If the experimental data is translated to a clinical setting it may indeed lead to change, albeit representing an enormous increase in cost and complexity of patient care in emergency department. An association between thrombolysis and (FND) may have the potential to alter the pathological chain of events leading to decompressive craniectomy.

Conclusion

In conclusion, it is hard to conceive a brain protective intrinsic circuit that is activated to render brain less susceptible to traumatic or ischemic events. More likely however, is that the findings of these experimental studies are the result of neuronal circuit hyperactivation. Perhaps, neurogenic neuroprotection circuitry has a physiological function related to the regulation of microvasculature and modulation of brain metabolism in order to promote a more receptive regional micro-environment and prevent neuronal excitotoxicity by ordinary stimulus. In contrast, the effect of hyperstimulation that is responsible for neurological preservation seems to be the inhibition of apoptosis and regulation of the inflammatory cascade. The stimulation of deep brain nuclei to obtain changes in ischemic brain lesions outcomes and to promote changes in regional metabolism heralds a new era in functional neurosurgery. However, further studies are needed to translate this experimental data to changes in clinical practice.

Acknowledgments

We acknowledge support from FAPESP (Sao Paulo State Foundation of Research Support) and CNPQ (Brazilian National Counsel of Technological and Scientific Development).

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