Cerebellum (2015) 14:15–18
DOI 10.1007/s12311-014-0603-2

REVIEW

Remote Degeneration: Insights from the Hemicerebellectomy Model

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Published online: 25 September 2014
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Abstract When CNS lesions develop, neuronal degeneration occurs locally but in regions that are remote, yet functionally connected, to the primary lesion site. This process, known as "remote damage," significantly affects long-term outcomes in many CNS pathologies, such as stroke, multiple sclerosis, and traumatic brain and spinal cord injuries. Remote damage can last several days or months after the primary lesion, providing a window during which therapeutic approaches can be implemented to effect neuroprotection. The recognition of the importance of remote damage in determining disease outcomes has prompted considerable interest in examining remote damage-associated mechanisms, most of which is derived from the potential of this research to develop innovative pharmacological approaches for preserving neurons and improving functional outcomes. To this end, the hemicerebellectomy (HCb) experimental paradigm has been instrumental in highlighting the complexity and variety of the systems that are involved, identifying mechanisms of life/death decisions, and providing a testing ground for novel neuroprotective approaches. Inflammation, oxidative stress, apoptosis, autophagy, and neuronal changes in receptor mosaics are several remote damage mechanisms that have been identified and examined using the HCb model. In this review, we discuss our current understanding of remote degeneration mechanisms and their potential for exploitation with regard to neuroprotective approaches, focusing on HCb studies.

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Keywords Remote damage · Autophagy · Apoptosis · Inflammation · Hemicerebellectomy

Introduction

When a focal brain lesion develops, the damage is not limited to the lesion site—degeneration occurs in regions that are remote but functionally connected to the primary lesion site. This phenomenon is known as "remote damage" [1].

The principal aspect that differentiates remote damage from primary damage regards the location and dynamics of the injury. By definition, focal brain injury is circumscribed within a well-restricted area. In contrast, remote damage involves multiple, noncontiguous sites that, due to functional links, receive death signals from the primary site by axons that are involved in the primary lesion or by changes in neuronal activity. Further, mechanisms of remote site degeneration remain active, and damage continues to progress for months after the lesion, when no further degeneration is observed in the primary injury. This prolonged activity is the basis for the exploitation of therapeutic windows, prompting the development of models to analyze remote damage mechanisms experimentally [2].

In this area, the hemicerebellectomy (HCb) paradigm has been proven to be a reliable and effective model for examining remote degeneration mechanisms and testing pharmacological approaches [2, 3]. We discuss the current data on remote damage, focusing on the molecular and cellular events in the HCb model.

Hemicerebellectomy: a Cerebellar Paradigm of Remote Degeneration

HCb is the surgical ablation of the cerebellar cortex—in which half of the vermis and one cerebellar hemisphere, including

the deep nuclei, are removed and the vestibular nuclei and all surrounding structures are spared. HCb affects all neurons of the contralateral inferior olive and pontine nuclei due to the direct axonal lesions and simultaneously deprives these nuclei of cerebellar inputs. Based on the unilaterality of the lesion and the nearly complete crossover of the cerebellar input-output organization, it is possible to study an intact and a lesioned cerebellar circuit in the same animal using this model—a patent advantage when morphological and physiological comparisons must be performed [2].

Remote Responses After HCb

Structural and Morphological Changes

Many studies have examined axotomy-induced remote degeneration in precerebellar neurons after HCb [4, 5]. Injured neurons undergo a series of morphological changes within days and weeks of development of the primary lesion, such as chromatolysis, downregulation of basophilic cytoplasmic substances, nuclear eccentricity, nuclear and nucleolar enlargement, cell swelling, and dendrite retraction [2].

This type of lesion induces extensive neuronal death in the olivary and pontine nuclei, which begins several days after the damage and can persist for approximately 2 months, during which the axotomized neuronal populations fade [4, 5].

Notably, degeneration-related phenomena do not occur during this time. At any given point, neurons assume various states of degeneration. Because axonal damage is induced at only one time, these disparities suggest differences in neuronal sensitivity and the dynamics of several reactive/compensatory mechanisms [4, 5].

Inflammation and Oxidative Stress

Inflammation has opposing functions in the damaged brain, providing neuroprotection and causing harm, depending on the context [6]. After HCb, this phenomenon is not limited to the primary lesion site and can involve remote regions [7]. In such areas of remote inflammation, microglial and astrocytic activation is evident by 7 days, plateauing at 3 weeks, and despite decreasing in intensity, this activation persists until 2 months after the injury [7].

As in primary lesion sites, glial activation influences remote degeneration by producing toxic mediators, such as pro-inflammatory cytokines, nitric oxide, glutamate, and free radicals [7]. However, although microglia and astrocytes are activated in remote regions after HCb, they have disparate functions. Astrocytes, but not microglial cells, release hazardous factors, such as IL-1β [7] and inducible nitric oxidase synthase (iNOS)-derived NO, which accelerate remote degeneration [8].

Oxidative and nitrosative stresses mediate the pathogenesis of several neurological diseases [9] and in remote degeneration after HCb [8]. In the HCb paradigm, oxidative/nitrosative stress results from a vicious cycle between axotomized neurons and reactive astrocytes, in which ROS that is released from injured neurons triggers chronic activation of iNOS in astrocytes. In turn, iNOS-derived NO diffuses to neurons and reacts with intracellular superoxide to form peroxynitrite. iNOS/NO, synthesized by activated astrocytes, diffuses in high concentrations into injured neurons, exacerbating axotomy-induced mitochondrial damage and leading to neuronal death. This crosstalk between neurons and glia establishes a perilous loop that accelerates and aggravates remote degeneration [8].

Apoptosis and Autophagy

Apoptosis is active during brain development and in virtually all conditions of brain damage [10]. It can be stimulated by two pathways: the intrinsic mitochondrial pathway and the extrinsic death receptor pathway. In the first, noxious stimuli target the mitochondria directly or through transduction by proapoptotic members of the Bcl-2 family, such as Bax and Bak [10]. In the second, cell surface receptors transmit apoptotic signals that are initiated by specific ligands, such as caspase-8, activating other caspases to orchestrate apoptosis.

Remote degeneration is primarily an apoptotic process that is regulated by the mitochondria [11, 12]. After HCb, axotomy death signals that are transported retrogradely to axotomized neurons primarily affect the mitochondria [11], inducing release of massive amounts of cytochrome c into the cytoplasm [11, 12], and to a lesser extent effects DNA fragmentation through caspase-3-dependent signaling, leading to remote cell death [11, 12].

Another mechanism of many CNS degenerative phenomena is autophagy, an evolutionarily conserved catabolic process that targets cellular components and organelles for physiological degradation [13]. Autophagy begins with the formation of double-membraned vesicles, which subsequently engulf cytoplasmic components, including cytosolic proteins and organelles, to become autophagosomes (APs). APs fuse with lysosomes to form autolysosomes, in which components are degraded by lysosomal hydrolases [13].

Dysregulation of this mechanism is involved in several diseases, including neurodegeneration [13]. Recently, autophagy machinery has been implicated in remote damage after HCb [14]. In this model, we have described the cascade of events that links the early stages of mitochondrial dysfunction to cell death, identifying the time frame during which neuronal autophagy is active. These results support the hypothesis that autophagy machinery is activated in response to mitochondrial sufferance and as a reactive mechanism that protects neurons by engulfing the damaged mitochondria, rendering it

essential for neutralizing proapoptotic factors and favoring internal homeostasis [12].

Further, the strict kinetics of the activation of autophagy after cytochrome c release from damaged mitochondria suggest that apoptosis begins only if the level of proapoptotic stimuli from damaged mitochondria exceeds their clearance by autophagy [12]. Nevertheless, this causal link between autophagy and apoptosis remains hypothetical.

Neurotransmitter Systems Activation and Interaction

Remote damage is the result of complex pathophysiological mechanisms that involve various neurotransmitter systems that operate independently, antagonistically, or synergistically to affect cell populations, based on data from the HCb model, demonstrating that many neurotransmitter systems are activated transiently in specific time frames and in certain neuronal populations. Of the systems that have been analyzed, the purinergic, nitrergic, and endocannabinoid systems are the most prominent systems to be implicated in remote damage. Morphological, molecular, and functional evidence suggests that the sustained activation of these systems is an endogenous reactive mechanism of axotomized neurons that sustains survival [2].

Positive and negative interactions between neuroprotective systems have been observed in the HCb model. The

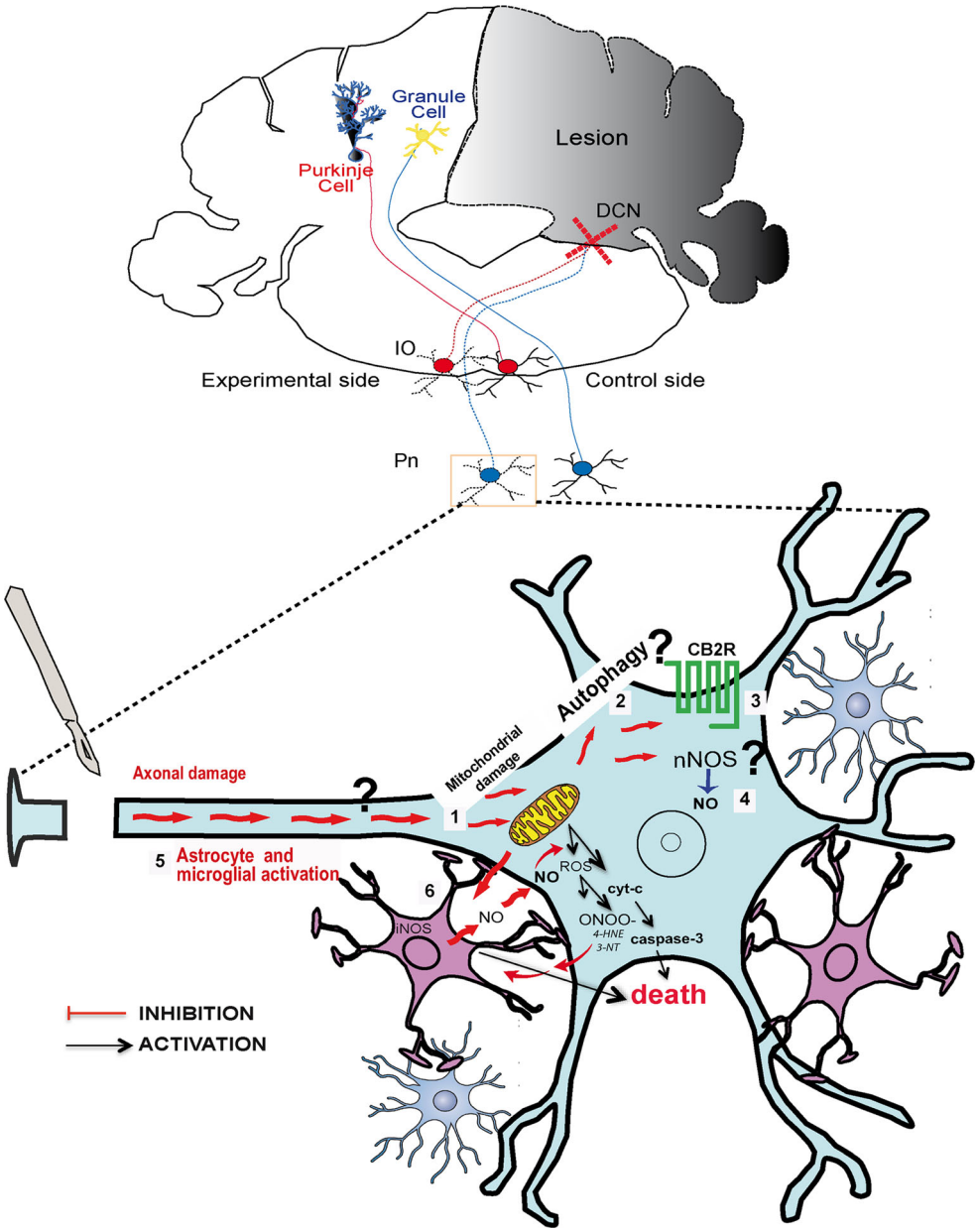

Fig. 1 Schematic of the main events intervening in remote damage after hemicerebellectomy (HCb). Due to the crossover of the cerebellar input-output organization, HCb induces axonal lesions and subsequent degeneration of the contralateral inferior olive (*IO*) and pontine nuclei (*Pn*), with sparing of the IO and Pn on the ipsilateral side. After damage, retrograde signaling reaches the cell body, provoking the activation of different events. Among these, mitochondrial damage and apoptosis (*1*), autophagy (*2*), cannabinoid 2 receptor (*CB2R*) (*3*) and neuronal nitric oxide synthase (*nNOS*) (*4*) modulation, astrocyte and microglial activation (*5*), and oxidative mechanisms (*6*) become active in specific time frames in sustaining remote damage

endocannabinoid and nitrergic systems cooperate in mediating remote neuroprotection. After HCb, pharmacological stimulation of the endocannabinoid system—specifically CB2R—modulates NO production, altering the balance between neuronal nitric oxide synthase (nNOS) and iNOS in remote areas, and improves cellular and neurological outcomes.

The nitrergic system does not interact solely with the endocannabinoid system. In HCb, nitrergic and purinergic interactions have been reported [5], suggesting a positive functional interaction between the two systems. Conversely, a negative interaction has been observed between endocannabinoids and glucocorticoids. The concomitant activation of both systems mitigates the neuroprotective effects of either system alone [15]. Thus, the characteristics of these examples of time-locked activation are key elements in planning neuroprotective strategies.

Conclusion

The significance of remote cell death in neurological disorders and in clinical outcomes is now well established. Based on its reliability and reproducibility, the HCb model has been proven to be a valid paradigm that can be used to study the mechanisms of remote degeneration and their significance in recovery after CNS injuries. With this model, we assert that remote damage is not the repetition of mechanisms at the primary lesion site on a smaller scale—rather, remote damage is a multifactorial phenomenon (Fig. 1), in which the various components of remote damage, including inflammatory, apoptotic, autophagic and oxidative mechanisms and neurotransmitter modulation, become active in specific time frames and can interact or compete in sustaining remote neuronal fate.

Acknowledgments This work was supported by the Italian Ministry of Health (Ricerca Corrente—MM), by the International Foundation for Research in Paraplegia (IFP) (MTV), and by the program Young Researchers of Italian Ministry of Health (GR10.184; MTV). The professional editorial work of Blue Pencil Science is also acknowledged.

Conflict of Interest The authors declare no conflict of interest.

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