Melanocortins Defend their Territory: Multifaceted Neuroprotection in Cerebral Ischemia

Stroke is the leading cause of adult disability and the third leading cause of death (1). Its dramatic, often catastrophic attack unleashes an extremely challenging therapeutic target. Typically, an intravascular blood clot occludes a vessel such as the middle cerebral artery, causing focal ischemia deep in its perfusion territory, and triggering a rapidly closing window of opportunity for treatment before neurological damage becomes irreversible. The sole approved therapy is to attempt reperfusion using thrombolytic agents within 3 h of symptom onset, but most patients are excluded by this requirement or by other eligibility criteria, and the treatment exposes patients to significant risk of intracerebral hemorrhage. Only a fraction of treated patients experience some benefit (2, 3). Consequently, a major goal of stroke research is to develop interventions that are effective and safe after longer treatment delays. Against this backdrop, in this issue Giuliani et al. (4) report promising findings of pharmacological neuroprotection in cerebral ischemia by a synthetic melanocortin (ACTH/MSH-related peptide).

Pathways of Destruction, Frustrated Interventions

The intracerebral events contributing to ischemia- and stroke-induced brain damage are dynamic and convoluted. Blood flow interruption leads to rapid depletion of energy substrates, failure of transmembrane ionic gradients, massive depolarization, and excessive release of neurotransmitters including the abundant excitatory transmitter glutamate, exacerbated by impaired reuptake mechanisms. This triggers cascades of interrelated, overlapping processes contributing to a rapid, early wave of excitotoxicity, involving excessive entry of Ca(2+)
 and other ions, cell swelling, activation of intracellular kinases and proteases, excessive production of reactive oxidative and nitrosative species (ROS, RNS), damage to cell membranes, and organelle failure. These events also promote an inflammatory response that begins within hours and develops more slowly. This involves up-regulation of chemokines and endothelial adhesion molecules including P-selectin and intercellular adhesion molecule-1 (ICAM-1), which promote tissue infiltration by leukocytes, endo

Melanocortins Protect the Postischemic Brain through Multiple Lines of Defense

After transient global cerebral ischemia in gerbils, produced by temporarily occluding both common carotid arteries—which mimics the sort of forebrain ischemia that follows cardiac arrest rather than stroke per se, but nevertheless triggers a spectrum of pathophysiological events similar in many ways to those that occur in focal ischemia—treatment with the melanocortin receptor (MCR) agonist, Nle4, D-Phe7-
α-MSH (NDP-MSH) reduced postischemic tissue injury and improved recovery of behavioral functions, even when treatment was begun up to 9 h after ischemia. There are five known MCR subtypes, common to humans and other mammals (designated MC1R through MC5R). The MC4R has been a focus of interest concerning its roles in controlling energy balance and is a potential target for new antiobesity drugs. In adult animals, it is principally expressed in the central nervous system (CNS), where it is the predominant known MCR subtype, and is expressed widely in neuroendocrine and autonomic centers as well as in basal ganglia, hippocampus, and cerebral cortex (12). The neuroprotective effects of NDP-MSH were prevented by a selective antagonist of MC4R (4), raising the possibility of targeting this multitaled receptor as a novel strategy for stroke pharmacotherapy.

Peripheral melanocortin treatment markedly inhibited biochemical and histopathological markers of ischemia-induced tissue damage and neuronal loss in the CA1 subfield of hippocampus, a region important in spatial learning and memory that is particularly sensitive to ischemia-induced cell death (5). Markers of hippocampal apoptosis were reduced, as was activation of caspase-3, an apoptosis-mediating “executioner” enzyme, and of the stress-activated protein kinases c-Jun N-terminal kinase (JNK) and MAPK p38 (p38), thought to be involved in activating caspasps and proinflammatory genes (5). On the other hand, ischemia-induced activation of two antiapoptotic enzymes, Bcl2 and BclXL, was enhanced (4), suggesting that activating MC4R up-regulates this putative endogenous neuroprotective pathway (13).

At a functional level, NDP-MSH treatment blocked the ischemia-induced impairment of spatial learning and memory for at least 12 d, perhaps reflecting in part the MC4R-mediated reduction of hippocampal cell death. Remarkably, the highest dose of NDP-MSH actually enhanced learning in nonischemic controls. The latter effect could involve established neurotrophic actions of melanocortins, including promotion of neurite sprouting and functional recovery from nerve injury (14). Earlier, a hypoxic-ischemic brain injury in rats was found to increase MC4R mRNA levels in the contralateral (uninjured) striatum (15), a region involved in movement planning and executive function, which is impaired after transient global ischemia (16). Such MC4R up-regulation might reflect a role of endogenous melanocortins in promoting adaptive transfer of functions to the undamaged hemisphere (15), consistent with the increased neural plasticity and functional reliance on the contralateral hemisphere that occur during stroke recovery (17). Hence, the present MC4R-mediated improvement of behavioral outcomes (4) may involve neuroprotective actions, regenerative trophic effects, or promotion of adaptive plasticity, in addition to suppression of damage pathways (Fig. 1).

**Real or Illusory Promise in this Line of Defense?**

Earlier disappointments notwithstanding, several facets of the new findings (4) suggest translational relevance for new stroke pharmacotherapeutic strategies. First, the favorable effects of melanocortins on diverse endpoints suggest they may target multiple cell types and processes contributing to postischemic cell death. For example, these results, along with earlier findings (18, 19), represent a logical application of the long-recognized suppressive effects of melanocortins on various proinflammatory pathways and on systemic post-ischemic injury (20). After transient focal brain ischemia, the native MCR agonist α-MSH suppressed the elevated TNF-α and IL1-β expression seen in the perfusion territory of the occluded middle cerebral artery, and reduced cortical/striatal TNF-α levels after global forebrain ischemia (19). Similar actions may contribute to reported protective effects of melanocortins in systemic and peripheral organ ischemia-reperfusion injury models. For example, α-MSH treatment reduced organ damage resulting from renal or splanchnic...
ischemia-reperfusion in rodents, by actions including reduced expression of ICAM-1, TNF-α, iNOS, and other leukocytic inflammatory markers, even when treatment was delayed for 6 h in the case of renal ischemia (reviewed in Ref. 20). Melanocortins also reportedly reduced injury and death in myocardial ischemia-reperfusion, while preventing the large surge in blood-borne free radicals (presumably ROS) occurring within 2 min of coronary reperfusion (20, 21), perhaps reflecting protection against mitochondrial and cell membrane failure.

The effectiveness of delayed NDP-MSH treatment in cerebral ischemia (4) is significant because researchers seek to extend the therapeutic window of stroke therapies to many hours after symptom onset. In this connection, blocking JNK (22) or p38 (23) activity in focal ischemia models with intracerebrally applied inhibitors dramatically reduced the resulting infarct volumes, motor impairments (22), or intracebral TNF-α, IL-1β, iNOS, and COX-2 expression (23), even when treatments were delayed 6–12 h, reflecting their roles in activating the delayed waves of inflammatory processes and cell death. NDP-MSH suppressed global ischemia-induced JNK and p38 activation after similar treatment delays (4), suggesting that this pathway could mediate the neuroprotective effects of delayed MC4R activation. Also, whereas the blood-brain barrier can present a major obstacle to prospective stroke pharmacotherapies, the efficacy of ip NDP-MSH treatment indicates that it had sufficient access systemically to the relevant MC4R-expressing cells, either within the brain parenchyma or in systemically exposed elements of the neurovascular unit. It is unknown whether nonneuronal elements of the neurovascular unit express MC4R, but melanocortins reportedly suppress NF-κB activation and expression of TNF-α and iNOS in activated microglia and peripheral macrophages, and also inhibited their induction within rat hypothalamus in vivo by actions attributed to MC4R (20, 24). Antipyretic actions of melanocortins could also potentially help protect against stroke-induced neural damage. Patients often present with or develop elevated body temperature after stroke, and even mild increments in body temperature exacerbate outcomes in animal models and clinical studies alike (5, 25). Both α-MSH and ACTH suppress fever, and in rats the antipyretic effect of α-MSH is mediated by MC4R, acting within the CNS (26).

Other factors also augur favorably concerning the feasibility of MC4R-targeted neuroprotective strategies. A nonselective MC3R/MC4R agonist peptide (PT-141) showed apparent safety and efficacy in clinical trials for treatment of erectile dysfunction (27). Like α-MSH and ACTH in animal studies (28), PT-141 had no significant effects on cardiac function and blood pressure (27)—a critical concern for prospective stroke treatments. Melanocortins also lack immunogenicity, which caused inflammation, adverse events and premature termination of clinical trials of a neutrophil-targeted monoclonal antibody in stroke patients (5). Also, treatment with a high dose of ACTH-(1–24), which is a nonselective agonist at other MCR subtypes in addition to its classic adrenocortical receptor, MC2R, reportedly had dramatic, life-saving effects in patients with aortic dissection, a form of hemorrhagic shock, associated with reduced plasma TNF-α levels and improved cardiovascular function (29). The mechanisms involved are not clear, but the authors found similar, adrenal-independent effects of ACTH in rat models of hemorrhagic shock, which they attributed to a descending vagal pathway, activated within the CNS by the MC4R (20, 30).

Elucidating the MC4R-expressing cells and downstream mechanisms that mediate the neuroprotective actions of melanocortins may prove challenging given their broad range of potential cellular and molecular targets within the neurovascular unit, perhaps involving overlapping or redundant pathways. An interesting question that arises is whether NDP-MSH acts directly on neuronal MC4R to suppress apoptosis, e.g. by up-regulating antiapoptotic enzymes or suppressing TNF-α or COX-2 expression, which are increased in injured neurons (5). The physiological role of the constitutively expressed MC4R in hippocampal and cortical neurons has been a conundrum, because these cells do not seem to receive significant innervation by melanocortin-producing neurons (31). Or, could posts ischemic MC4R activation engage networks of intracerebral, neuroprotective neurons—analogous to a recently described descending vagal cholinergic antiinflammatory pathway (32), thought to suppress peripheral inflammatory responses, TNF-α expression, and ischemia-reperfusion injury by activating cholinergic receptors on tissue macrophages?

Whether MC4R-directed neuroprotective strategies will show further promise toward new stroke therapies is an open question. Given the wealth of positive indicators and the seeming absence of contraindications, this seems an inviting problem for interdisciplinary attack, and an opportunity to assess the translational potential of the widely reported and multifaceted antiinflammatory actions of melanocortins (20), by developing sufficient preclinical evidence to determine whether clinical trials of such therapies are warranted in this desperate patient population.

Jeffrey B. Tatro, Ph.D.  
Division of Endocrinology, Diabetes, Metabolism and Molecular Medicine  
Tufts-New England Medical Center and Tufts University School of Medicine  
Boston, Massachusetts 02111

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Address all correspondence and requests for reprints to Jeffrey B. Tatro, Ph.D., Division of Endocrinology, Diabetes, Metabolism and Molecular Medicine, Box 268, Tufts-New England Medical Center, and Tufts University School of Medicine, 750 Washington Street, Boston, Massachusetts 02111. E-mail: jbtatro@tufts-nemc.org.

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