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Neuroprotection by endogenous and exogenous PACAP following stroke☆

Yun Chen [a], Babru Samal [a], Carol R. Hamelink [a], Charlie C. Xiang [b], Yong Chen [c], Mei Chen [b], David Vaudry [a], Michael J. Brownstein [b], John M. Hallenbeck [c], Lee E. Eiden *

[a] Section on Molecular Neuroscience, Laboratory of Cellular and Molecular Regulation, NIH, Bethesda, MD, 20892, USA
[b] Laboratory of Genetics, National Institute of Mental Health, NIH, Bethesda, MD, 20892, USA
[c] Stroke Branch, National Institute of Neurological Diseases and Stroke, NIH, Bethesda, MD, 20892, USA

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Abstract

We investigated the effects of PACAP treatment, and endogenous PACAP deficiency, on infarct volume, neurological function, and the cerebrocortical transcriptional response in a mouse model of stroke, middle cerebral artery occlusion (MCAO). PACAP-38 administered i.v. or i.c.v. 1 h after MCAO significantly reduced infarct volume, and ameliorated functional motor deficits measured 24 h later in wild-type mice. Infarct volumes and neurological deficits (walking faults) were both greater in PACAP-deficient than in wild-type mice, but treatment with PACAP reduced lesion volume and neurological deficits in PACAP-deficient mice to the same level of improvement as in wild-type mice.

A 35,546-clone mouse cDNA microarray was used to investigate cortical transcriptional changes associated with cerebral ischemia in wild-type and PACAP-deficient mice, and with PACAP treatment after MCAO in wild-type mice. 229 known (named) transcripts were increased (228) or decreased (1) in abundance at least 50% following cerebral ischemia in wild-type mice. 49 transcripts were significantly up-regulated only at 1 h post-MCAO (acute response transcripts), 142 were up-regulated only at 24 h post-MCAO (delayed response transcripts) and 37 transcripts were up-regulated at both times (sustained response transcripts). More than half of these are transcripts not previously reported to be altered in ischemia.

A larger percentage of genes up-regulated at 24 hr than at 1 hr required endogenous PACAP, suggesting a more prominent role for PACAP in later response to injury than in the initial response. This is consistent with a neuroprotective role for PACAP in late response to injury, i.e., even when administered 1 hr or more after MCAO. Putative injury effector transcripts regulated by PACAP include β-actin, midline 2, and metallothionein 1. Potential neuroprotective transcripts include several demonstrated to be PACAP-regulated in other contexts. Prominent among these were transcripts encoding the PACAP-regulated gene Ier3, and the neuropeptides enkephalin, substance P (tachykinin 1), and neurotensin.

Keywords: Pituitary adenylate cyclase activating polypeptide, PACAP; PACAP-deficient mouse; Middle cerebral artery occlusion, MCAO; Neurological severity score, NSS; Cerebral ischemia; Infarct volume; cDNA microarray; PACAP responsive gene; Neuroprotection; Neurotrauma

1. Introduction

Pituitary adenylate cyclase-activating polypeptide (PACAP) was discovered as a member of the secretin/glucagon/vasoactive intestinal peptide (VIP) family [1]. It is widely distributed in neurons of the brain and peripheral nervous system [2], and has multiple transmitter and trophic functions [3]. PACAP affects neuronal cell cycle exit during central nervous system formation [4], promotes neuronal differentiation in cultured rat sympathetic neuroblasts [5,6], differentially modulates proliferation of central and peripheral neuroblasts [7], stimulates neuritogenesis in PC12 cells [8,9] and regulates neuron-specific gene expression in human neuroblastoma cell lines [10]. PACAP prevents apoptotic cell death and protects cultured rat cortical neurons against glutamate-induced cytotoxicity [11], and dopaminergic neurons against 6-hydroxydopamine–induced cytotoxicity [12].

These properties of PACAP are consistent with a possible endogenous neuroprotective role after stroke or brain injury. In fact, PACAP has significant neurotrophic and neuroprotective

☆ Data deposition: The raw data from the expression profiling experiments reported in this paper have been deposited in the Gene Expression Omnibus database (accession no. GSE5902). The GSE5902 will be hyperlinked when it is released.

* Corresponding author. Tel.: +1 301 496 4110; fax: +1 301 402 1748.
E-mail address: eidenl@mail.nih.gov (L.E. Eiden).

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effects in brain damage models *in vivo* [13,14] and *in vitro* [15–19]. PACAP prevents the ischemic death of rat CA1 neurons when given either intracerebroventricularly or intravenously in a model of transient global ischemia, even if administration is delayed for 24 h after the ischemic event [13]. PACAP can prevent loss of hippocampal neurons even after systemic administration presumably because it is a ligand of peptide transport system PTS-6, which transports it across the blood-brain barrier (BBB) at modest rates [20]. Systemic administration of PACAP also effectively reduces infarct volume in a rat model of focal ischemia when administration begins 4 h after MCAO [14]. In addition to its neuroprotective effects, PACAP is cardioprotective for cultured ischemic myocytes [21], attenuates reperfusion injury following ischemia of brain, kidney and lung [22–24], and is protective in endotoxemia *in vivo* [25], all suggesting an even more general role for PACAP in injury response.

Cerebral ischemia causes neuronal cell death in the areas where blood flow to the brain is permanently or transiently interrupted, and additional neuronal cell death (secondary damage) in immediately surrounding brain areas, due to altered extracellular ion concentrations, release of excitotoxic neurotransmitters such as glutamate, and elevated levels of toxic cytokines and generation of reactive oxygen species through inflammatory processes that begin shortly after an ischemic event [26,27]. No pharmacological treatment is available to prevent these post-ischemic events that occur as a consequence of the initial injury. Thus, investigation of PACAP's role in the prevention of secondary neuronal damage in ischemia is potentially of great importance.

Characterization of changes in gene expression that occur during stroke, and therapeutic intervention in stroke, can illuminate mechanisms of ischemic neuronal death and neurological dysfunction, or identify novel therapeutic targets in cerebral ischemia. The sequencing of the mouse and human genomes, growing databases for differential gene expression in different tissues and under different conditions in each species, and annotation of human and mouse mRNA transcripts, have contributed in concert to this process. Many genes have been reported to be differentially expressed and highly up-regulated in cerebral ischemia [28–38]. Some of the cognate encoded proteins may contribute to the pathogenesis of cerebral ischemia [39]. Microarray analysis offers a unique way to investigate changes in gene expression over time, identify the transitional transcriptome involved in a given process, and potentially evaluate the efficacy of treatments aimed at abating neurological deficits. This technique has been recently applied to identify genes associated not only with nervous tissue response to cerebral ischemia, but in related conditions such as spinal cord and traumatic brain injury in which secondary neuronal damage plays a key role in the long-term physiological outcome of the initial injury [40–43].

We report here that PACAP is neuroprotective in a mouse model of cerebral ischemic damage. We compare the therapeutic effects of exogenous PACAP in improvement in neurological function and reduction of infarct volume of the ischemic brain, with the effects of endogenous PACAP deficiency on exacerbation of ischemic damage and functional outcome of cerebral ischemia. Comparison of transcriptome alterations during ischemic insult in wild-type and PACAP-deficient mice provides a basis for identification of mRNA transcripts whose regulation by PACAP may be related to its neuroprotective effects. PACAP may act in part through the enhanced expression of other neuropeptides in ischemic cortex, including met-enkephalin, substance P, and neurotensin.

2. Materials and methods

2.1. Animals

A mouse strain deficient in the expression of PACAP was employed, as described previously [44]. Adult 129XC57BL6 PACAP -/- and +/+ F2 littermates were used in this study, and maintained with a standard 12-h light/dark cycle with humidity and temperature controlled at normal level, and water and food available ad libitum. All experiments were approved by the Animal Care and Use Committee of the National Institute of Mental Health Intramural Research Program.

2.2. Middle cerebral artery occlusion (MCAO)

Animals were anesthetized with 5% isoflurane for induction and 1.5% isoflurane for maintenance in a 30% O_2 and 70% N_2O gas mixture via a face mask. Body temperature was maintained in the normal range (36 °C–37.5 °C) with a heating pad during the operation. MCAO was performed as described [45]. Briefly, a 1.5-cm skin incision was placed between the left margin of the orbit and the tragus. The temporalis muscle was incised and retracted to expose the squamous portion of the temporal bone. A small burr hole (2 mm) was made with a high-speed microdrill through the outer surface of the semitranslucent skull over the visibly identified middle cerebral artery at the level of the inferior cerebral vein. The inner layer of the skull was removed with fine forceps, and the dura was opened with a 30-gauge needle. The left MCA was electrocauterized between the olfactory tubercle and the distal segment of the MCA (unilateral occlusion) using a small vessel cauterizer. The olfactory branch of the MCA, which was consistently present, was preserved. The coagulated MCA segment was then transected with microscissors to verify that the occlusion was permanent. The surgical site was closed with 4-0 monofilament nylon sutures. A single dose of antibiotics (Gentamicin, 2.5 mg/kg) was applied topically before closing the surgical sites, and 30% lidocaine cream was also applied to all surgical sites as an analgesic. After surgery, mice were returned to their cages and allowed free access to food and water. Duration of anesthesia did not exceed 30 min.

2.3. Drug administration

Administration of PACAP began 1 h after MCAO. PACAP (Phoenix Pharmaceuticals, Inc., Belmont, CA) was dissolved in 0.9% saline containing 0.1% BSA. For i.c.v. administration, a